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CONTENTS OF VOL. 36, NO. 4

STUDIES OF THE CLOVE TREE

I. SUDDEN-DEATH DISEASE AND ITS EPIDEMIOLOGY

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(With Plates 8 and 9 and 8 Text-figures)

Sudden-death disease of cloves has been present and steadily increasing in both Zanzibar and Pemba for many years. The only premonitory symptom is a slight chlorosis followed by thinning of the foliage and reduction of the absorbing system. Death follows after a period, which may vary from only a few days to many months. Death occurs from lack of water caused by the disorganization of the absorbing roots.

The outbreaks fall into three classes: (1) the sporadic, which ceases to spread after killing a few trees; (2) the 'Pemba' type showing clear peripheral spread; (3) the diffuse epidemic type.

In Pemba some 500 small outbreaks are scattered through the clove areas and some seem to be passing from type (2) to type (3). The total number of trees affected there is less than in Zanzibar, where the situation approximates to a single outbreak involving half of the clove-growing area of the island. The rate of spread of the disease varies, but it is accelerating.

Various causes, physical, physiological, and pathological, have been suggested to account for the condition. The epidemiology suggests that all but a pathogenic hypothesis can be discarded. Of the possible pathogens, a virus carried by a lethargic vector is the most probable. Suspicion is attached to a scale insect which is tended and transported by the red tree-ant, *Oecophylla longinoda*.

INTRODUCTION

Although in some years Madagascar contributes appreciably, most of the world's clove supply is produced in the two largest islands, Zanzibar and Pemba, of the Protectorate of Zanzibar, which is situated just south of the equator off the east coast of Africa.

For more than fifty years, the deaths of clove trees in Zanzibar have been reported, but it is only during the last decade that these are known to have been due to three main causes. One is physiological, due to changing water-table, when all trees in a certain area die simultaneously. The second is known as die-back, where the branches of the tree concerned die progressively over a number of years. Its exact cause is unknown, but it is probably due primarily to bad husbandry and unsuitable conditions. The third, and now far the most important cause is 'sudden-death'. Its cause is uncertain, but it is devastating the plantations of Zanzibar Island, and increasing in Pemba, thereby forming a most serious threat to the clove industry

* Seconded from Rothamsted Experimental Station.

and to the whole economy of the Protectorate. It is also present in Madagascar, although as yet it does not seem a serious problem there. So far as we know, sudden-death is absent from the Seychelles, where a few cloves are grown, from Ceylon, India, and Penang, and also from the Moluccas where the clove is indigenous.

Little has been published about the disease, although work has been carried on at intervals for many years. Use will be made in this paper of records in various departmental files, and reference made to an occasional unpublished report.

Historical

Although deaths of clove trees from unknown causes have been recorded in Zanzibar since 1892, it was not until 1907 that McClellan (1923) first suggested the presence of a specific disease. Some of these deaths were, in all probability, the early stages of the present epidemic.

It was suggested in 1907, and again in 1910, that sudden-death was probably of fungal origin, and in 1914 Dowson, then Mycologist in British East Africa, was asked to visit Zanzibar and report. He blamed adverse soil conditions. Welsford (1922) reached the conclusion that sudden-death was caused by a specific fungal pathogen, which she thought to be a *Peziza* sp. The fungus was, however, never isolated, and no transmission work was done. Troup (1932) suggested that the larvae of a cockchafer might cause sudden-death, but Kirkpatrick (1932), showed that they do not attack the clove.

In 1934, Wigg deduced that the disease was due to a root parasite, but Campbell (1940), who worked on the disease for a year, failed to demonstrate the presence of any fungal, bacterial, or protozoal parasite and ascribed death to physiological causes. No further research was done after his departure, partly because of the war, and partly because of the belief, based on the failure to isolate a pathogen, that the disease would not become epidemic. However, further heavy losses occurred between 1939 and 1945 and research started again in 1947.

In Madagascar Fauchère (1907), reported that 'apoplexie' (which both Findlay and Campbell consider identical with sudden-death) was due to a fungus or an eelworm. Although Fauchère claimed to have found fungus in the roots, none was isolated or identified. Heim & Bouriquet (1937, 1938), like Campbell, deduced that the disease must be physiological.

The symptoms of the disease

The main characteristic of the disease is, as its name implies, suddenness. A tree showing no obvious signs of debility can, and often does, pass from apparent health to death in a few days. Although to the casual observer the name is justified, it is, nevertheless, misleading.

The first symptom of sudden-death is a very slight chlorosis, but as other causes often give rise to a similar effect, this is not diagnostic. In a good plantation where the disease is already present, an experienced observer is rarely at fault, but if

a proportion of the trees is unthrifty, no definite diagnosis can be made until a much later stage.

We have failed to find any early symptoms other than this slight general chlorosis. After this the disease takes a course which varies widely between two extremes. The first is generally, but not invariably, shown when young trees (say up to 20 years of age) are attacked. The tree declines very gradually, often taking 6 or more months. The leaves become more and more chlorotic, and the older ones absciss earlier than usual; this may continue until all are gone and the tree dies. More normally, however, the tree wilts before all the leaves have fallen, and those remaining dry on the tree and remain there.

The second course is the spectacular one from which the disease takes its name. After the slight and almost imperceptible premonitory symptoms (which may persist for many weeks) a rapid leaf-fall sets in, producing in the most acute form a heavy carpet of green and yellow leaves. This is accompanied by a slight general wilt, which rapidly increases until abscission is prevented and the remaining leaves dry on the tree, becoming a bright russet red and forming a conspicuous spectacle. From the onset of leaf-fall to death may be only a few days.

Available descriptions indicate that most deaths have approximated to the second type. Campbell speaks of the 'carpet' of green and yellow leaves, as though it were normal, while the general acceptance of the word 'sudden' is not without significance.

During 1948 we have found the more usual mode of death to lie between the two extremes, but tending toward the first. Examples of the 'sudden' have been seen, but comparatively rarely. The climatic conditions this year may have favoured the slower mode of death, but we are inclined to believe that the disease has changed character. Often (except when the tree dies gradually) an occasional branch remains green and unwilted after all the others are dead. Where the tree possesses vertical shoots these invariably behave in this way, but they eventually die too.

The time between the first diagnosis and the death of the tree is in all forms of the disease very variable, and to some extent reflects the weather. Many trees which we had marked as suspects assumed an appearance of health during the heavy rains of the south-west monsoon. When conditions again became dry they soon lost their healthy appearance, and death usually followed rapidly. This temporary recovery is in all probability due to the more readily available water supply. The fact that a branch from a wilting tree will recover when removed and placed in water and can be kept alive and apparently healthy for some time after the death of the tree, supports this view.

Since the final symptoms seem merely to be those of acute water shortage, and can be reproduced by cutting a branch and letting it wilt in the sun, attention is naturally directed to the root system. All investigators have reported that loss of absorbing roots accompanies the disease. By the time the final wilt sets in, the absorbing roots are almost absent, although the main framework of the root system seems to be healthy.

Both Storey (1940) and Campbell experimented with the root system. Reduction of absorbing area, whether by severe root pruning or by removing the soil from the roots without otherwise injuring them, does not produce the sudden-death picture, even when the treatment is severe enough to produce flagging and leaf-fall. The trees eventually recover. But ring-barking, although it produces little visible effect for 2 or 3 months, then kills the tree with symptoms indistinguishable from those produced by sudden-death. That the final symptoms are associated with water shortage is shown by measurements of the diurnal water deficits of leaves just before the onset of the final wilt. They are much higher than those of leaves from healthy trees. Work now in progress indicates that this is not so in the early stages of the disease.

EPIDEMIOLOGY

Sudden-death has been considered to be a disease of mature or over-mature stands, and senility has been suggested as a possible cause. While there is every reason to believe that sudden-death was never confined to mature trees, there seems to be no doubt that it rarely attacked young ones in the past. This is no longer true. Trees of all ages are affected, although losses of young trees are much less than those of older ones. In a plantation of trees of mixed ages, young trees often die equally with older ones, especially where sudden-death is very active, such cases being illustrated in Text-fig. 2 and Pl. 9, fig. 2. However, they may die a few weeks later than the mature ones. Plantations consisting entirely of young trees are rarely attacked, although at least one is at present being destroyed by epidemic sudden-death. It is not yet known whether seedlings of 2–3 years of age are affected, but mortality among them is often high; and in the present state of our knowledge it is not always possible to determine the exact cause of death.

Confusion has also arisen since more than one cause can operate to give rise to symptoms very similar to sudden-death. Because of this, its incidence was thought to be very variable, and very heavy losses in certain years were confidently ascribed to it. Although Campbell did not see devastation in a peak year, he correctly deduced that such deaths were the result of a rising water-table, but wrongly considered them to be examples of sudden-death, produced by an edaphic cause.

Briant (1946) worked in areas in which such widespread deaths were occurring. By making detailed studies of the movement of the water-table and of the root systems, he found that flooding caused destruction of the roots. He thus confirmed Campbell's deductions as to the cause of death, but was able to show that the epidemiological picture produced when a group of trees is killed in this manner differs from that characterizing a sudden-death outbreak, even though the death symptoms of the individuals are very similar. This work is important because it enables the few isolated 'peak' years to be eliminated from consideration.

Three main types of outbreak can be distinguished. We believe the differences between them to be more apparent than real, and that all intergrades can be found, but the following subdivision is convenient.

The sporadic outbreak

This type seems at one time to have been common in Zanzibar. It arises at a distance from others, and after having involved a dozen or so trees ceases to spread further. In many older clove plantations isolated patches occur which are bare of clove trees or occupied by young ones. Local report often states that these gaps were caused by sudden-death, and Campbell considers that in all probability all were so caused. It is impossible to say of any present outbreak that it is of this type.

The epidemic outbreak with peripheral spread (Pemba type)

This type is common in Pemba, and rare in Zanzibar. The zone of demarcation between diseased and apparently healthy trees is narrow, and the disease is therefore advancing peripherally. This kind of outbreak often kills all the trees in its path, irrespective of age, leaving a completely cleared patch (Pl. 8, fig. 2 and Pl. 9, fig. 1). Text-figs. 1 and 2 illustrate typical examples. The first is a small outbreak, very recently started. The second is from the edge of a large one, and illustrates the almost diagrammatic nature of the advance, and also the way in which young trees die more or less simultaneously with the older ones as the advancing wave of death passes over them.

This type frequently gives rise to smaller outlying ones, and these, spreading in their turn and often fusing, sometimes produce a compound outbreak of great complexity (Text-fig. 3). It is difficult to interpret, and it is not possible to say whether more than one primary focus was involved. Whether or not the older gaps in the original stand, obviously cleared by disease and now in process of regeneration, were all derived from one focus, it is clear that a number of recent smaller outbreaks have arisen, and of the ultimate fate of all the mature trees in the vicinity we have no doubt whatever. This is illustrative of a tendency for the more complex Pemba-type outbreaks to approximate to the third type.

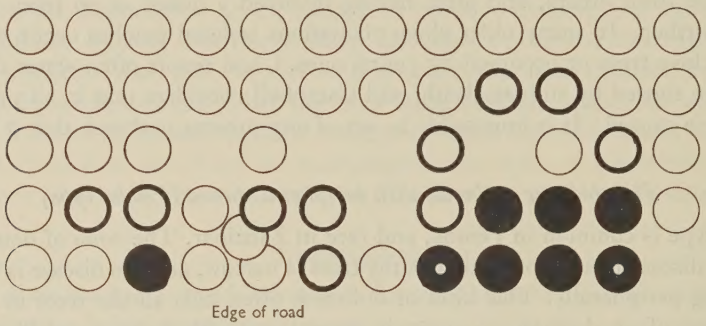
The diffuse epidemic outbreak. Zanzibar type

The term outbreak should not properly be applied to most of the sudden-death areas in Zanzibar Island, for, as will appear later, they are really active zones bordering on devastated areas. They are characterized by a high death-rate and a moderately even incidence of death over a wide area. That this apparent type of spread is deceptive will be shown later.

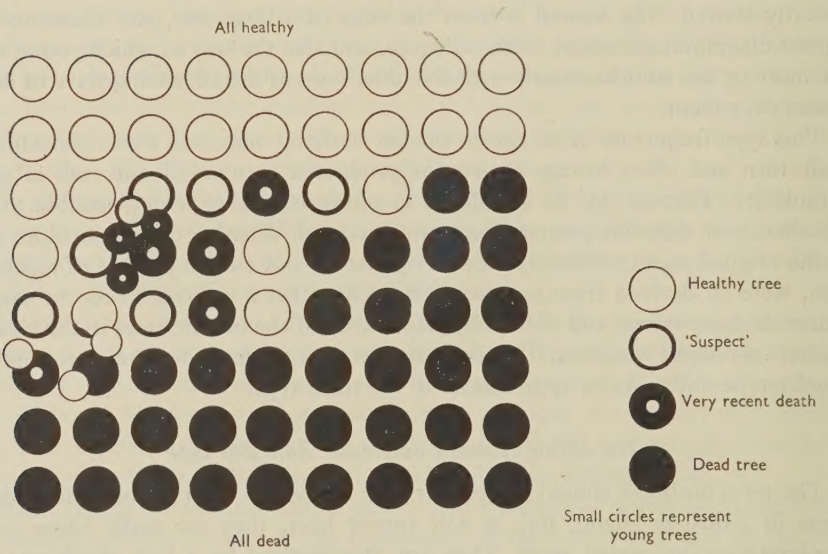
Records are available from two sources of the progress of the disease in the active zones. The first is death-rates of a block of trees at Machui over a period of 11 years, the second of 500 trees at Selem over $3\frac{1}{2}$ years. These deserve fairly detailed treatment.

(1) *The Machui Block.* This block of 800 trees has been recorded at monthly intervals since 1934. Sudden-death is reported to have begun in 1933, and by the

time recording started there had been 6% casualties. Text-fig. 4 illustrates the development of the disease in this block. Most of the stand consisted of trees aged about 60 years, but there were seventy younger trees of which forty-three were



Text-fig. 1. Outbreak at Piki, Pemba.



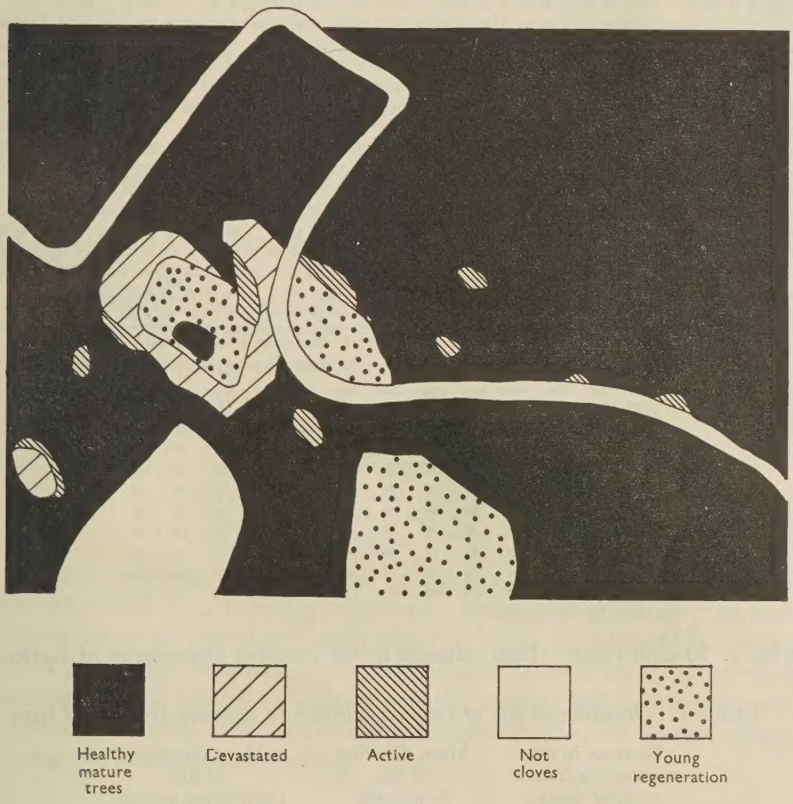
Text-fig. 2. Part of edge of outbreak at Migorani, Pemba. The small circles represent young trees, perhaps 15 years old. The main stand is not less than 50 years old, and probably much more.

estimated to be 12–24 years of age, and thirteen aged 4–12 years. Campbell interprets this to mean that sudden-death had caused these casualties over the past 25 years, spreading very slowly and not 'flaring up' until 1933.

Dr Yates at Campbell's request studied the distribution of the early casualties, and concluded that they were not random, and that there was a definite tendency

for sudden-death trees to occur together, and for areas heavily affected at the earlier dates to continue to show mortality.

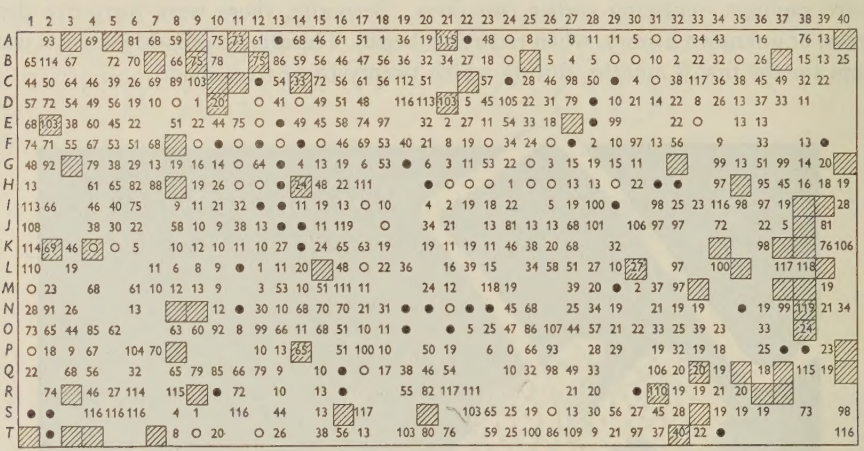
Three factors seriously reduce the value of these recordings. First, seventy trees were saplings, replacing earlier casualties. Secondly, there were forty-five dead trees, of unknown history, when recording began. Thirdly, no monthly records were kept for the first 8 months, during which twenty-three trees died.



Text-fig. 3. Sketch-map of outbreak at Chunguziko, Pemba.

It is of some interest, however, to consider the duration of life of the trees in relation to their distance from the original deaths, as Dr Pereira has done at our request. For this estimate the four outer rows on all sides of the block are discarded, since quite probably there were dead trees just outside the block. The mean duration of life of all trees whose deaths were individually recorded, after May 1935, is shown in Table 1. The data suggest a rate of spread of two tree intervals every 9 months, but the 30 months average life of group I cannot be accounted for on this hypothesis.

The table does show quite definitely that the expectation of life of a clove tree in a sudden-death area varies directly with its distance from the early casualties.



▨ Original poles and saplings

● Deaths prior to September 1934

○ Deaths September 1934 to April 1935 inclusive

Deaths	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1935					1	2	3	4	5	6	7	8
1936	9	10	11	12	13	14	15	16	17	18	19	20
1937	21	22	23	24	25	26	27	28	29	30	31	32
1938	33	34	35	36	37	38	39	40	41	42	43	44
1939	45	46	47	48	49	50	51	52	53	54	55	56
1940	57	58	59	60	61	62	63	64	65	66	67	68
1941	69	70	71	72	73	74	75	76	77	78	79	80
1942	81	82	83	84	85	86	87	88	89	90	91	92
1943	93	94	95	96	97	98	99	100	101	102	103	104
1944	105	106	107	108	109	110	111	112	113	114	115	116
1945	117	118	119									

Text-fig. 4. Machui Block. (Data collected by the Zanzibar Department of Agriculture.)

TABLE 1. *Duration of life of trees in relation to distance from dead trees*

Distance in tree intervals from original deaths	Mean duration of life in months	Mean duration of life (combining groups)
1	30·2	30·2
2	39·3	39·3
3	39·2	
4	49·4	48·4
5	47·4	

(2) *The Selem Block.* This block of 500 trees has been recorded by the Department of Agriculture from 1942 to 1946. Unfortunately, recording did not start until nearly 20% of the stand was dead. The death-rate was so high that when recording was discontinued 3 years later, over 80% of the trees were dead.

Both blocks show considerable variations in the monthly death-rate, and there is close parallelism between the variation in the death-rate at the end of the Machui recording and that at the beginning of the Selem recording. The correlation between these variations is significant to the 5% point and almost to the 1% point. The correlation between the annual death-rates is even more marked, being significant to better than the 1% point.

The question immediately arises as to the cause of this variation. It has long been held that the drier periods of the year are those during which most deaths occur. But the correlation between monthly rainfall and death-rate is not significant, although Campbell showed there was a tendency for the deaths in any month to be negatively correlated with the rainfall 4 months earlier. This correlation did not reach the 5% point.

Sudden-death affects the tree for a long time before the onset of the final symptoms which are retarded, although not stopped, by an increase in the supply of water to the tree. A fairly prolonged period of damp and dull weather will be more effective for this purpose than will brief periods of heavy rain. To test this, correlations were worked out for the blocks at Selem and Machui.

x = monthly death-rate as a percentage of the stand at the time.

y = number of rainy days per month.

r = rainfall during month.

z = number of months from start of recording.

	Selem	Machui
$r_{xy.rz}$	−0·283	−0·293
$r_{xr.yz}$	−0·042	−0·084
$r_{xz.yr}$	0·363	0·513

$r_{xy.rz}$ in both instances is just on the borderline of significance, $r_{xr.yz}$ is clearly insignificant, while $r_{xz.yr}$ is highly significant. Bearing in mind that the monthly death-rates are in all probability determined by other factors, and no more than influenced by climatic factors, a higher partial correlation is hardly to be expected.

The correlation of death-rate with time is very marked and very important. It is in complete conformity with the evidence presented in the next section, where it is shown that the disease is accelerating.

Development of the disease in Zanzibar and Pemba

The two islands are geologically distinct, Zanzibar being part of the mainland, and Pemba oceanic. Both are ridge-backed and neither rises to more than 400 ft. While Zanzibar is gently undulating, Pemba is highly dissected and hilly. The soils of the clove-growing districts of Pemba are fairly uniform and are more fertile than those of Zanzibar (Calton, 1948). Rainfall varies in different parts of both islands, but it is on the whole higher in Pemba than in Zanzibar.

Cloves were extensively planted in both islands in the early 1800's, but those of Zanzibar were largely replanted immediately after a hurricane in 1872, which

uprooted most of the trees. Except for a strip along the east coast, almost the whole of Pemba is planted with cloves, but in Zanzibar, clove growing is mainly confined to about one-quarter of the island, to the north-west.

The distribution of sudden-death now appears strikingly different in the two islands. In neither can its incidence, or rate, or plan of spread, be correlated with soil conditions, topographical position, or rainfall. It is correlated with soil fertility only to the extent that spread is often more rapid in a good stand.

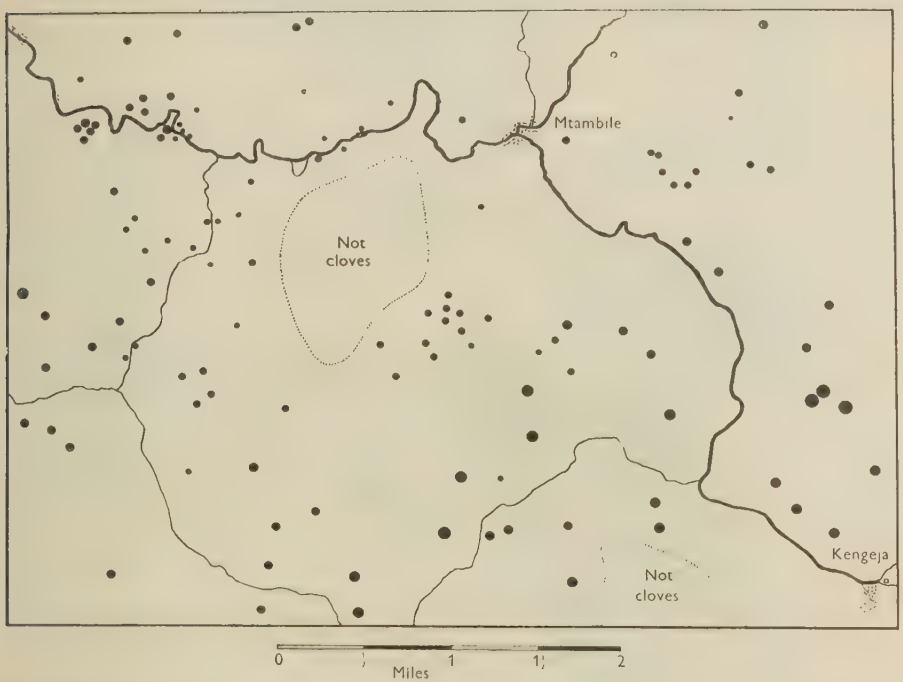
Text-fig. 5. Map of southern half of Pemba showing outbreaks discovered by reconnaissance survey in 1948. (Prepared by D. Winter, Zanzibar Department of Agriculture.)

Present situation in Pemba

Our knowledge of the present situation in Pemba is mainly due to a survey carried out in 1948 by the Zanzibar Department of Agriculture, during which some 500 outbreaks were visited. It is known that this survey is not complete, and that many of the smaller, and perhaps some of the larger, outbreaks have escaped attention.

We have studied representative outbreaks from every part of the island, and have mapped forty-three of them.

No part of Pemba is free from sudden-death, the recorded outbreaks being evenly distributed. Text-fig. 5 shows the southern half of Pemba and illustrates this. Attempts have been made to utilize air-survey photographs to expand results from ground survey, which unless organized on a scale at present impracticable must necessarily be incomplete. This presents considerable difficulties. Many, perhaps most, outbreaks can be identified with certainty, especially where dead and dying



Text-fig. 6. Map of rectangular area indicated in Text-fig. 5, showing outbreaks visible in air-survey stereo-pairs, 1947. (Prepared by D. Winter, Zanzibar Department of Agriculture.)

trees can be recognized on the periphery. In Pemba, most trees are felled as soon as they die and are used for fuel; a small gap in the canopy is all that can be seen from the air. Consequently there is some uncertainty with regard to a proportion of the data thus obtained.

Text-fig. 6 has been prepared from air-survey stereo-pairs taken in 1947; many more outbreaks than were discovered by ground survey are shown. This is partly due to the fact that complex outbreaks can often be separated into their components

by study of air photographs, and partly because of the admitted incompleteness of the ground survey. Most Pemba outbreaks are small, averaging less than fifty trees each, but some are very large, and up to a mile in length. The latter often have a history of sudden-death extending over 20 years or more, as can be seen by the different ages of replanted trees. Active disease in these very large old outbreaks is often limited to one or more spots on the periphery. Others are both large and active, some showing a death-rate of over 300 trees in the past 5 years (Pl. 8, fig. 2). One large and active outbreak is at present advancing at the rate of one row every 6 weeks (Pl. 9, fig. 1). Still others begin to show the phenomenon characteristic of Zanzibar, that of being bounded by an active zone, and not by a plain periphery.

Present situation in Zanzibar

Viewed on a large scale, Zanzibar appears now to contain but a few very large outbreaks. The disease, after having devastated perhaps half of the whole clove-growing area, is advancing northwards on an undulating front, stretching almost the whole width of the clove belt. The active peripheral zone is often several hundreds of yards wide. A few outbreaks are in advance of this front, and a number of areas left by the first wave of disease are now being attacked; as are also some regenerated parts of the devastated area.

Text-fig. 7 is a diagrammatic map illustrating the widespread destruction, bordered by active zones, characteristic of the present stage of the Zanzibar epidemic. This was prepared from aerial-survey photographs taken in August 1947. These are often difficult to interpret, but the situation as shown is compatible with what was to be seen from the ground in 1948. We have tried to illustrate the various zones as defined below. No great accuracy is claimed, for, except in a few places, the boundaries between them cannot be defined.

Legend to Text-fig. 7

Text-fig. 7. Sketch-map of part of Zanzibar Island showing the distribution of the disease in August 1947.

Devastated. Although in many parts of the area every tree has been killed, complete destruction is not here implied, but rather destruction as an economic unit. Often islands of apparently healthy trees can be found in a devastated zone, also islands of trees being attacked by the disease. Where these are well-marked they are indicated diagrammatically.

Active. This can vary from a widespread 'peppering' of the area concerned, with small groups of dying trees, as in the early stages of the Machui epidemic, to cases where most of the trees are dying.

Healthy. Apparently free from disease. Some idea of the speed of this advance can be obtained from points *A–D* marked on the road, although the disease tends to advance in different places at different speeds. In 1939 it had reached *A*, and by December 1946 had advanced 4 miles to *B*. By December 1947 it had moved 300 yards to *C*, and in another ten months advanced again 310 yards to *D*.

Text-fig. 7.

Development of the disease in the two islands

Little is known of the early incidence of the disease, partly because of the confusion which existed prior to Briant's work, and partly because there seems always to have been a tendency to discount sudden-death as being relatively unimportant.

Welsford (1922) wrote that she was told by the Arabs, that 'even in the far-off days of their grandfathers, when slave labour was abundant, clove trees would die suddenly without apparent cause, just as they do now'. This is in agreement with Fitzgerald (1895), who speaks of groups of dead trees in 1893 (slavery was abolished in 1897). At that time the plantations of Zanzibar would be about 20 years old. Possibly due to this age difference, and the consequent relative closeness of the canopy in Pemba, the early incidence of the disease seems to have been greater there, since McClellan called attention to the dangers of the disease, especially in Pemba, in 1907 and again in 1910. Welsford states that although the disease was serious in both islands by 1922, it was worse in Pemba.

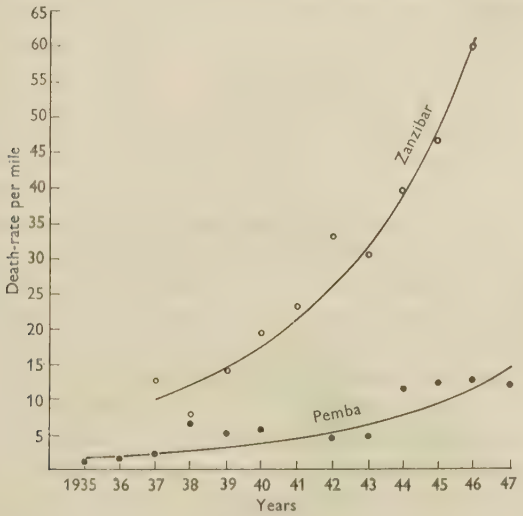
Some time after 1922 the disease increased rapidly in Zanzibar. We have been unable to discover any records by which the early stages of the present Zanzibar epidemic can be reconstructed, but some little evidence is available on the incidence of death. The most important source is the records of replantings. The Zanzibar Government has established clove nurseries throughout both islands, and issues seedlings free of charge to 'approved' planters, and for a nominal sum to others. In addition an unknown, but appreciable number from other sources is planted, generally with volunteer plants from under-mature trees. New plantations are rare, and it is safe to say that the enormous number of seedlings planted in recent years has not increased the total area under cloves. Two seedlings are normally planted per site, and the death-rate of these in the field may be as high as 30%. In Zanzibar, almost all regeneration is a direct result of sudden-death. In Pemba, perhaps one-quarter of all plantings is the result of die-back, and the remainder is due chiefly to sudden-death.

The assumption that the number of sudden-death casualties in any one year equals one-quarter of the seedling issues in Pemba, and one-third of those in Zanzibar, is probably conservative, for it ignores the appreciable number of self-sown seedlings utilized; and furthermore, the assumption that all young trees which die in the field are replaced is not wholly justified.

Text-fig. 8 represents the death-rates for each island. It will be seen that, especially in Zanzibar, the increase in death-rate with time approximates to an exponential curve. The variability of the Pemba data may be partly due to the general poverty of the clove growers, who only regenerate when they can afford the costs of replanting. Little information on the actual losses in either island can be found. Campbell conducted a partial census and estimated annual death-rates as being nearly 6000 for Zanzibar and nearly 4000 for Pemba, over the period 1935–9 inclusive. Campbell's data were obtained by inspecting all plantations whose owners

reported the presence of the disease. As it is certain that only a proportion did so, his results (although valuable as the only factual data available on an island-wide basis) are an underestimate.

Some large plantations in Zanzibar are Government property, and a census of standing trees was taken in 1930, and again in 1940. The groups of plantations concerned were being attacked, and it seems likely that the results shown in Table 2 somewhat exaggerate the average death-rate.



Text-fig. 8. Death-rate estimated from numbers of seedlings issued for regeneration

TABLE 2. *Losses of mature trees in plantation groups*

	Machui and Dunga	Selem	Kizimbani
1930 census	27,717	12,894	27,916
1940 census	24,233	11,730	17,795
Loss	3,484	1,164	10,121
Percentage loss	12·5	9·0	36·6

From various observations carried out from the air in 1947 and from the air and ground in 1948, we consider that at least half of the mature clove trees on Zanzibar Island have died during the past 10 or 12 years. Perhaps 20 or 30 years ago a number of scattered outbreaks, possibly of the Pemba type, represented the stage of the disease in Zanzibar. These have now coalesced in part, to form what is virtually one complex outbreak covering about half of the clove-growing belt, and steadily advancing northwards. We consider that, in a relatively short time, all the mature clove trees in the island will have succumbed. Of the ultimate fate of the large

number of seedlings and young trees which have been planted in the wake of the disease, it is as yet too early to form a definite opinion, but some are already being attacked.

Possibly due to the spectacular development of the disease in Zanzibar Island, the potential danger existing in Pemba seems to have been overlooked until 1948, when data collected by ourselves, the Department of Agriculture, and the native staff of the Clove Growers Association, revealed a disturbing situation. The rapidity of spread in some of the 500 outbreaks there, and the similarity of some to the Zanzibar type, makes us fear that the recent history of Zanzibar may shortly be repeated in Pemba.

Discussion

A large number of suggestions have been made to account for sudden-death. We consider the epidemiological picture we have presented to be totally incompatible with any physical or physiological cause. The method of spread, and the increasing death-rate is, we think, evidence that some form of pathogen is involved. This view was held by Briant (1946) and by Posnette (1948). The former says: 'It is my opinion that whatever other factors contribute towards an unhealthy state of the trees, the view that a pathogenic organism is responsible for sudden-death should not yet be discarded.' We therefore dismiss all non-pathogenic hypotheses and propose to discuss possible pathogens, in the light of such evidence as is available.

The ultimate death of the tree is from water shortage, and a cut branch from a wilting tree will regain its turgor and will remain alive and fresh for some days when placed in water. Injecting water into a branch still attached to a dying tree will keep it alive and green until after the rest of the tree is dead. This water shortage is obviously the direct consequence of the death of the absorbing roots, which are desiccated by the time that definite and final leaf-symptoms set in. Death proceeds from the periphery and the main roots are healthy and unrotted at the time of death. Attention is directed to causes which could produce this result.

(1) *Direct attack on the absorbing roots by a pathogen.* Welsford (1922), on microscopical evidence, held that the death of the roots was caused by a fungus which she considered to be a species of *Peziza** but it was never isolated, and in view of later results, need not be further considered. Campbell studied the fungi invading the root system, and found that a *Melanconium* species was, as he describes it, 'in the forefront of the invaders'. He thinks that, in all probability, this fungus was Welsford's *Peziza* sp. Briant has also isolated it. Campbell found it wherever dead or dying rootlets occurred, in healthy and unhealthy trees alike, but considered it most unlikely that sudden-death could be caused by an attack of soil pathogens.

An additional argument is based on the fact that seedlings of various ages are often found under dying trees. Furthermore, it has been Zanzibar practice for some

* Welsford gave this fungus the manuscript name of *Peziza caryophyllata* but, as she gave no diagnosis and the work was unpublished, the name is *nomen nudum*.

years past to interplant dying plantations with seedlings. Consequently there are many thousands of young trees planted with their roots in intimate association with those of dying trees. Yet it is exceptional for any of these to die at the same time as, or even shortly after, the older ones.

The foregoing arguments, of course, are general and not specific to any soil pathogen. So far as the main groups of pathogens are concerned, Campbell was unable to demonstrate the presence of eelworms or bacteria and we have confirmed his findings. An occasional eelworm has been found, and bacteria occur in rare swollen roots, but both are equally prevalent in sudden-death areas and in plantations remote from it.

Campbell considered that the fungi he found were ubiquitous and non-pathogenic. We find occasional fungal hyphae in roots of healthy and sudden-death trees alike, but there is no evidence of any such attack as would be expected of a virulent pathogen.

(2) *Attack on the conducting tissues by a pathogen.* Any cause which will stop the translocation of carbohydrates to the roots will kill the clove tree with sudden death symptoms. This has been demonstrated by both Storey and Campbell, and confirmed by us. A clove tree when ring-barked shows little signs of shock at first, but the foliage soon begins to thin. Death in the sudden-death manner takes place some 3–4 months after ringing. As would be expected, starch accumulates above the ring and none is to be found in the roots. This directs attention to a pathogen which can affect translocation.

Storey, in 1938, drew attention to the similarity between sudden-death and phloem necrosis of coffee in Surinam, as reported by Stahel. Campbell investigated this possibility, and was unable to establish any evidence in its favour. Not only were no flagellates demonstrated, but phloem abnormalities such as are associated with flagellosis in coffee could not be found. Our findings confirm Campbell's.

Occasional fungal hyphae are found in the phloem of diseased trees and also, although more rarely, in healthy ones. Campbell considered the possibility that a phloem fungus was concerned, and sampled, by an increment borer, twenty-four diseased and seven healthy trees, taking a total of 928 cores. He used 605 of these for the isolation of fungi, and the remainder for microscopical study. 68% of his samples from sudden-death trees, and 7% from healthy trees, contained fungi which could be grown in culture, but in only 25% of his samples was he able to demonstrate fungus microscopically. The mycelium, therefore, even when present, would appear to be scanty. Campbell says, 'in fact, no extensive attack of the phloem could be detected anywhere in the main laterals or trunk. I regarded these results as indicating that fungi present in the outer bark rapidly established themselves there after the tree wilted.'

The last three words indicate quite clearly that Campbell's 'diseased trees' were trees which were moribund. They had been subjected to an increasing water strain for possibly many months, and were 'advanced cases'.

This, in our view, adequately explains the larger proportion of cases in which fungal hyphae occurred in the 'diseased' trees. The fungi concerned were of five types, of which two were relatively common. Campbell attempted to infect trees by culturing the fungus on pieces of clove root and inserting the root in borings in the trunks of healthy trees, but without result. In an attempt to show whether fungal toxins were responsible, he placed cut branches in liquids in which the fungus had been cultured, and also injected such liquids into branches of healthy trees. His results were all negative.

These negative results confirm deductions on other grounds. Interruption of carbohydrate supply to the roots by anything which interferes with translocation, e.g. ringing, must result in starch accumulation above the ring, and starch depletion below it. Campbell studied starch distribution in diseased trees, and could find no evidence of any accumulation whatever. Neither can we. On this evidence alone it seems most unlikely that any localized interruption to the translocation of elaborated materials can be admitted.

(3) *Virus*. That virus infection is responsible must now be considered. This possibility was recognized in the late 1930's both in East Africa and in England, and was supported by Nutman (1946) and Posnette (1948). Not only does none of the evidence conflict with this view, but in its symptom picture, pattern of spread, and increasing death-rate, sudden-death offers many parallels to known virus diseases, such as swollen shoot of cacao, some peach diseases, phloem necrosis of elm, quick decline of citrus, etc. Moreover, at least one apparent anomaly can be explained on the hypothesis of a virus transmitted by a lethargic vector. This is the relative insusceptibility of young trees. With such a vector, other things being equal, rate of spread would be expected to be proportional to the closeness of the canopy, and to the actual size of the trees where these are not in contact. Furthermore, in the early stages of an epidemic, small trees might legitimately be expected to escape infection altogether. This is what is found in the field.

Healthy trees are probably more susceptible than those in indifferent health, another characteristic of virus attack. The variation in symptom picture and speed of death is not contrary to a virus hypothesis, for this can be explained by the existence of more than one virus, of strains of a virus, by differences in the environmental conditions, or in the state of the tree at the time of infection. As young trees usually die slowly, the latter would seem the more probable explanation, although several causes might operate.

Of the time elapsing between infection and death we can give no estimate. In one particular outbreak trees are now dying regularly at 6-week intervals (see Pl. 9, fig. 1). In this plantation the trees form a closed canopy, with every tree in close contact with its neighbour; for a vector attacking foliage or stems they must form a single habitat. The 6-week interval might be considered as being the time necessary for a tree to become systemically infected, i.e. the period between a tree becoming infected by its neighbour on one side, and itself becoming infective on the other.

Variations in rates of spread may also be correlated with variations in insect populations, and the different development of the disease in Zanzibar and in Pemba may be explicable on these grounds.

We have searched for possible vectors and have found only one, a scale insect, sufficiently numerous to be considered as a possible vector.* It is cultivated and transported by the red tree ant, *Oecophylla longinoda* (Latreille) var. *textor* (Santechi). The relations between the ant and the scale are both interesting and suggestive.

The ant is widely distributed in Zanzibar and Pemba. In almost all plantations in Zanzibar it is present in such great numbers as to render picking the clove crop an unpleasant, and sometimes an almost impossible, task, for the ant's bite is severe and it attacks with ferocity. In Pemba, on the other hand, although locally numerous, it usually occurs in much smaller numbers.

Nests are built by fastening leaves together with silk derived from the larvae, and from them the ants forage over the tree and the surrounding ground, carrying prey to the nest. Insects of all types are captured, including spiders, wasps and bees. The ants on any one tree, or on any group of trees whose canopies are in contact, appear to form one community; they ferociously attack ants from other trees, and resist colonization from other communities.

The ant cultivates the scale inside the nest, where it is often present in enormous numbers. It also establishes strong infestations on the young terminal twigs, especially on young inflorescences. These colonies are always invested in a fine silken tent which is probably a protection against at least two parasites† of the scale. Apparently in the absence of the ant, these parasites gain the upper hand, and so far as our observations go, the ant is never found on the clove without the scale, nor the scale without the ant.

When a tree dies from any cause, the ant colonies disperse. When sudden-death is the cause there is a marked tendency for them to leave just before the wilt stage, but the ants in adjacent trees sometimes force them to remain until later. When they leave, the scales are carried away and used to found new colonies at considerable distances from the old home, sometimes hundreds of yards away. Mature clove trees are chosen where possible, and young cloves, or indigenous vegetation are avoided; but where mature clove trees are rare, or where strong hostile communities make them difficult to colonize, ants have been observed to make use of a wide variety of plants, including young cloves.

All the phenomena at present associated with the epidemiology of sudden-death disease are in conformity with the hypothesis that the scale is the vector and that it is usually carried from plant to plant by the ant. We have never found sudden-death, either in Zanzibar or in Pemba, without finding also the ant and its associated

* Since this paper went to press, we have had the advice of Dr W. Hall, Director, Commonwealth Bureau of Entomology. On a short visit to the Protectorate, he found several coccids on the clove but agreed that only one could be regarded as a possible vector. This, he considers, is probably a new species of *Saissetia*.

† Dr Hall finds these are probably undescribed species of *Encyrtus* and *Coccophagus*.

scale. Furthermore, any sucking insect, unless it is one protected by the ant, would have difficulty in surviving on a tree which is constantly being foraged by numerous, carnivorous ants, which even manage to catch winged insects such as bees and butterflies. Thus probable vectors seem likely to be restricted to the coccids or aphids.

The rapid spread in closed canopies; the slower spread in areas where the canopy is opened by branch-breaking or die-back, or where the trees are small and isolated; the relative freedom of young trees; the fact that in some outbreaks in Zanzibar (and a few in Pemba) a proportion of old trees escape attack and often do not succumb until much later; the marked difference between the epidemiology of the disease in Zanzibar and in Pemba, are all readily explicable.

Parts of Zanzibar are inhabited by an exotic terrestrial ant, *Anaplolepis longipes* (Jerdon), which attacks the red tree ant. Colonies of this move (amoeba-like) over parts of the island, but, although it has been present for at least 50 years, it appears to have moved on a radius of only about 6 miles. One particular plantation was freed from the tree ant by this predator about 18 months ago, according to native report, and at least 12 months ago according to our own observations. So far as we can determine, the plantation is free from scale, which disappear in the absence of their associated ant, and the predator is still in occupation. The fact that deaths still continue might be explained by the period which must elapse between infection and death. On the assumption that the scale is the vector, this period is at least 12 months.

Work is in hand, the results of which should confirm or refute the hypothesis outlined above.

We have great pleasure in acknowledging the help given by the Director of Agriculture, Zanzibar, who has allowed us free access to departmental records, and has permitted the publication of data taken from them.

Our thanks are also due to the Director of Agriculture, Kenya, and to the Director of the West African Cacao Research Institute, for permitting visits from Dr R. Le Pelley, Senior Entomologist; Dr H. C. Pereira, Soil Chemist; and Mr A. F. Posnette (Pathologist). The help which these officers have given is gratefully acknowledged.

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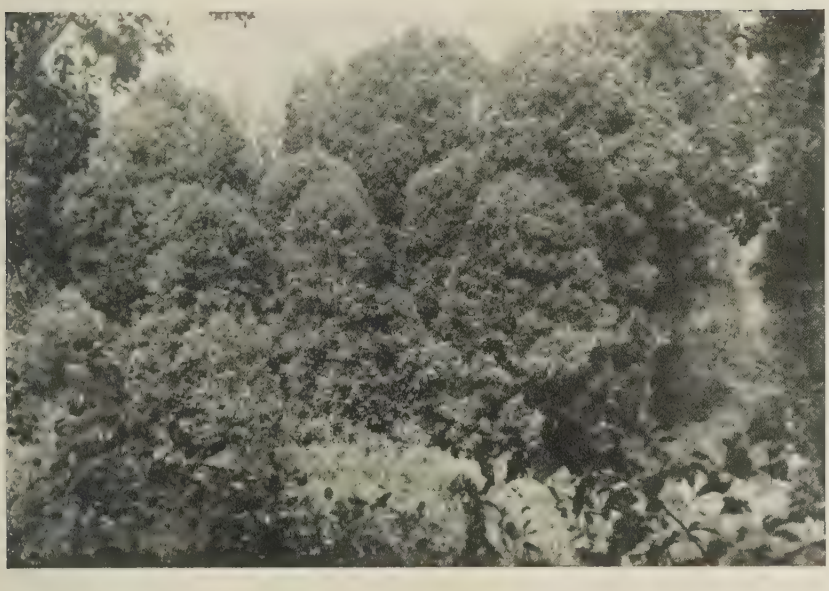

Fig. 1

Fig. 2

NUTMAN AND SHEFFIELD—*Studies of the clove tree*

Fig. 2

Fig. 1

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EXPLANATION OF PLATES

Plate 8

Fig. 1. Group of healthy clove trees, Pemba.
Fig. 2. Edge of outbreak in Pemba looking from the centre of the devastated area. Stumps and young replants are in the foreground. Dead and dying trees are to be seen on the periphery, with apparently healthy trees beyond.

Plate 9

Fig. 1. Edge of outbreak showing sharp line of demarcation between dead trees on the right and apparently healthy ones to the left. This particular outbreak is advancing at the rate of one row every 6 weeks.
Fig. 2. Young clove tree which has recently died from sudden-death. The dead trees of the devastated area are to be seen in the background. The trees above and behind the camera are still apparently healthy, and this accounts for the absence of vegetation in the foreground.

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VIRUS DISEASES OF CACAO IN WEST AFRICA

IV. EFFECT OF VIRUS INFECTION ON GROWTH AND WATER CONTENT OF CACAO SEEDLINGS

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Apparently healthy cacao seedlings were compared with those infected before planting with 'swollen shoot' viruses. The leaf area and the fresh and dry weights of each organ were measured. Infected plants were lower in dry weight, leaf area, relative growth rate and net assimilation rate; a smaller proportion of the dry matter was in the leaves and lateral roots, a larger proportion in stems and tap roots. Infection caused extensive necrosis of the lateral roots, and reduced the rate of depletion of reserves in the cotyledons and the water content of the plant. Many of these effects were apparent within a month of infection and planting.

In the course of investigations of the 'swollen shoot' virus diseases of cacao, Mr A. F. Posnette observed that the roots of infected seedlings were affected at about the same time as leaf symptoms appeared. Microscopical examination confirmed that the proportion of necrotic fine roots was much higher than in healthy seedlings of the same age. There is much poorer development of laterals in infected seedlings; some may be dead, others may be thick and pale with necrotic patches at the base; thick laterals of this type are characteristic of infected plants and absent from normal ones in which the root system has been undamaged. A series of experiments was planned to test quantitatively the effects of virus on early seedling development.

Experimental procedure

Embryos from which one cotyledon had been removed were infected by the bean-feeding technique of Posnette & Strickland (1948). Ten mealybugs (*Pseudococcus njalensis* Laing) were transferred from an infected plant to the embryo and allowed to feed for 24 hr.; they were then removed, and the embryo was planted in soil in a seed-box. Up to 150 embryos were planted at one time, and the plants were grown in shaded insectaries, the light intensity in which was about one-quarter of full daylight. Experiments were made with the virulent virus 1A (Posnette, 1947) except nos. I, IV and VI, in which the milder viruses from Olanla (Nigeria), Konongo and Mampong (Gold Coast) respectively were used. After a period of 25–78 days after planting, seedlings which had developed leaf symptoms and others which were apparently healthy were harvested. The roots were separated at the cotyledonary node, carefully cleaned under water, and the laterals were separated from the tap root. The cotyledons (if still present) and all leaves were removed from

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the stem. Except in one experiment, all the leaves from a single seedling were combined to give one sample; their area was determined by the method previously described (Goodall, 1947, 1949). The fresh weight of each organ from each seedling was determined separately; the organs were then dried in a steam oven for not less than 24 hr. and the dry weights were determined.

In Exps. I–VI, all embryos planted were infested with vectors, and the comparisons made were between seedlings in which symptoms had developed and others in which none was evident (called 'controls'). Most of the latter were probably non-infected, but in a few—particularly in Exps. IV–VI, in which the seedlings were harvested before the second 'flush' of leaves had expanded—it may be presumed that the virus was present but latent. In Exps. VII and VIII every third embryo planted was uninfested, though otherwise treated in the same way as the infested embryos, and the uninfested seedlings were used as controls to compare with those infested and showing symptoms.

In Exp. VIII, two successive harvests were taken 29 and 43 days after planting. At the second harvest, a separate sample of seedlings was collected in which symptoms had developed since the first harvest; these are indicated in the Tables as 'L' (latent infection).

Significance of differences between infected and uninfected seedlings was tested by analysis of variance, variation between seed-boxes being eliminated. Where appropriate, and where the necessary measurements had been made, the harvest data were corrected for differences in embryo weight before planting.

Results

(*a*) *Plant dry weight.* The means for infected and control seedlings are given in Table 1. In every comparison, except Exp. VI with the mild Mampong virus,

Table 1. *Mean dry weight (mg.) of infected and control seedlings*

Experiment	Date of harvest	Days since planting	Mean dry wt./plant		s.e. of means
			Infected	Control	
I	6. ii. 47	78	648	923	57**
II	12. ii. 47	58	556	601	38
III	19. ii. 47	69	888	1169	49**
IV	25. ii. 47	43	617	665	30
V	31. iii. 47	32	502	510	12
VI	12. iv. 47	29	474	464	17
VII	19. vii. 47	25	501	512	5
VIII	5. ix. 47	29	566	609	10**
VIII	19. ix. 47	43	639	757	10***
VIII (L)	19. ix. 47	43	727		13*

* $P=0{\cdot}01-0{\cdot}05$. ** $P=0{\cdot}001-0{\cdot}01$. *** $P=0{\cdot}001$.

the dry weight of the infected plants was less than that of the controls, and in three experiments the difference was highly significant. In Exp. VIII a significant

difference became apparent after only 29 days, and in Exp. VII after only 25 days the small difference observed (2%) was almost significant. Exp. VIII showed that this difference increased significantly between 4 and 6 weeks from planting, the growth rate being much greater in the control than in the infected seedlings. The mean relative growth rates over this period were respectively 1·67 and 0·94%/day. The infected plants, 4 weeks after planting (Exps. V–VIII), had a dry weight some 2% below the controls; at 6 weeks (Exps. IV, VIII) this difference had increased to 12%; and after 10 weeks (Exps. I, III) to 27%. Thus, though no fully valid test is possible, it appears likely that the discrepancy in relative growth rate persists at least up to 10 weeks from planting; the fact that apical die-back is usually a later symptom suggests that it not only persists thereafter, but is accentuated.

In Exp. VIII, at 6 weeks the seedlings with latent infections were found to have a lower dry weight than the controls, though much larger than the seedlings in which symptoms were already visible at 4 weeks. In fact, the difference at 6 weeks between controls and seedlings with latent infections was similar to that at 4 weeks between controls and seedlings with overt infections. This suggests that the difference in dry weight begins to develop only after the appearance of foliar symptoms.

(*b*) *Dry weight of organs.* For each plant, the dry weight of each portion into which the plant had been divided was calculated as a percentage of that of the plant as a whole. The means for infected and control plants in each experiment are shown in Table 2.

The leaves of infected plants usually had a lesser proportion of the total dry weight and the stems a greater proportion than the controls. The cotyledon dry weight as a percentage of the plant was consistently higher in infected plants, suggesting that the infection interfered with the translocation of reserves. The proportion of material in the tap roots was greater in the infected than in the control plants after 8 weeks from planting. Some of the results at earlier stages of development show differences in the opposite direction, but at the time of harvest these plants (unlike those of the first three experiments) still retained their cotyledons, and the tap-root weight as a percentage of that of the plant, excluding the cotyledons, would show no significant difference. Most often the lateral roots were proportionately smaller in the infected plants, thus confirming the visual observations which led to this series of experiments; this difference, however, did not become evident until 6 weeks or more after planting, some 3 weeks later than the development of leaf symptoms.

When the results are considered on an absolute instead of on a proportional basis, the only occasions on which the dry weights of organs of infected plants are significantly greater than those of the controls are the cotyledons in Exp. VII (250 and 221 mg. respectively; S.E. = 6 mg.) and the stems of seedlings with latent infection in Exp. VIII (93 and 79 mg.; S.E. = 2 mg.). In most other comparisons, including the stems in Exps. I and III (which formed a greater proportion of the dry weight in the infected than in the control series), the absolute dry weight of the

organs of infected plants was less than that of corresponding organs in the control seedlings.

TABLE 2. *Mean dry weight of organs as percentage of plant in infected and control seedlings*

Organ	Experiment	Days from planting	Mean dry wt. as percentage of whole plant: Infected	Control	S.E. of means
Leaves	I	78	51·7	59·9	1·5**
	II	58	58·9	64·3	0·5***
	III	69	49·6	54·0	1·5
	IV	43	51·6	53·2	1·4
	V	32	38·2	40·2	0·9
	VI	29	34·5	36·6	1·1
	VII	25	24·2	29·2	1·0***
	VIII	29	33·5	35·3	1·1
	VIII	43	43·1	41·6	1·1
	VIII (L)	43	42·0		1·3
Stems	I	78	16·0	14·9	0·6
	II	58	14·4	12·5	0·4**
	III	69	15·5	14·6	0·4
	IV	43	9·5	9·9	0·4
	V	32	9·3	9·1	0·4
	VI	29	10·6	9·5	0·4
	VII	25	7·3	7·0	0·2
	VIII	29	7·9	6·6	0·2***
	VIII	43	11·2	10·5	0·2*
	VIII (L)	43	12·8		0·3***
Tap roots	I	78	29·7	22·1	1·3**
	II	58	24·9	20·3	0·7***
	III	69	30·5	25·1	1·5*
	IV	43	18·4	17·0	1·2
	V	32	21·6	21·8	1·1
	VI	29	17·1	17·2	0·5
	VII	25	15·2	17·4	0·4***
	VIII	29	13·6	18·0	0·7***
	VIII	43	19·1	22·3	0·7**
	VIII (L)	43	18·8		0·9**
Lateral roots	I	78	2·6	3·1	0·2
	II	58	1·8	3·0	0·2**
	III	69	4·4	6·3	0·6*
	IV	43	2·6	2·9	0·3
	V	32	2·3	2·4	0·2
	VI	29	1·5	1·4	0·1
	VII	25	3·3	3·0	0·3
	VIII	29	2·5	2·2	0·2
	VIII	43	2·5	3·3	0·2**
	VIII (L)	43	3·4		0·2
Cotyledons	I, II, III	Cotyledons shed			
	IV	43	18·0	17·1	1·1
	V	32	28·5	26·5	1·7
	VI	29	36·4	35·3	1·8
	VII	25	50·0	43·4	1·0***
	VIII	29	42·6	37·9	1·2**
	VIII	43	24·1	22·4	1·2
	VIII (L)	43	23·4		1·6

* P=0·01–0·05. ** P=0·001–0·01. *** P<0·001.

(*c*) *Leaf area.* The mean total leaf areas in each series are shown in Table 3. Infection has consistently decreased leaf area. In the samples harvested about 4 weeks from planting this decrease averaged about 14%, at 6 weeks 18%, and at 10 weeks 33%. In other words, the difference in leaf area increased during development in much the same way as that in plant dry weight, though at 4 weeks it was more marked. The plants with latent infections in Exp. VIII have as low a leaf area as those in which symptoms had been visible at the first sampling.

TABLE 3. *Mean total leaf area of infected and control plants*

Experiment	Days from planting	Mean total leaf area (sq.cm.) Infected	Control	S.E. of means
I	78	137	235	13***
II	58	138	176	9*
III	69	155	234	11***
IV	43	113	138	8
V	32	71	74	6
VI	29	67	74	4
VII	25	66	89	3***
VIII	29	86	101	5
VIII	43	120	147	5**
VIII (L)	43	118		7**

* $P=0{\cdot}01-0{\cdot}05$. ** $P=0{\cdot}001-0{\cdot}01$. *** $P<0{\cdot}001$.

TABLE 4. *Mean water content of infected and control plants*

Experiment	Days from planting	Mean water content (percentage of dry matter) Infected	Control	S.E. of means
I	78	272	291	9
II	58	313	332	13
III	69	257	287	6**
IV	43	272	277	11
V	32	275	283	15
VI	29	310	306	12
VII	25	351	394	6***
VIII	29	293	293	6
VIII	43	270	323	6***
VIII (L)	43	278		7***

* $P=0{\cdot}01$–$0{\cdot}05$. ** $P=0{\cdot}001$–$0{\cdot}01$. *** $P<0{\cdot}001$.

The data for Exp. VIII enable net assimilation rates to be computed for the period between 4 and 6 weeks from planting. The usual formula was applied, in spite of its disadvantages in relation to a plant expanding its leaves in 'flushes' (Goodall, 1950), and gave the figures of 0·0357 and 0·0606 g./sq.dm./week for the infected and control series respectively.

(*d*) *Water content.* Table 4 shows the mean water content of the whole seedlings, expressed as percentage of dry matter.

In all except Exp. VI, with the mild Mampong virus, the mean water content of the infected plants was less than that of the controls, though the magnitude of the

difference varied considerably. In Exp. VIII, the water content of infected seedlings decreased between 4 and 6 weeks, while that of the controls increased.

This difference in water content between infected and control seedlings occurred similarly in leaves, stems and tap roots; the lateral roots did not show such a difference, which is perhaps hardly surprising in view of the experimental errors involved in determining their fresh weight. The cotyledons harvested in Exp. VIII at 43 days showed a particularly marked difference in water content (infected 146%, control 261%, S.E. 12%), suggesting that senescence was more advanced in the infected plants and abscission more imminent.

Discussion

From their effects on leaf area or chlorophyll content it seems highly probable that many plant viruses reduce photosynthetic efficiency. Ainsworth & Selman (1936) found that the dry weight of young tomato plants was reduced by inoculation with tobacco-mosaic virus, a difference being evident within 2 weeks from inoculation, but the reduction was small compared with that of virus 1A on cacao growth.

Few measurements of the reduction in leaf area due to virus have been published. Stone (1936) found the average total leaf area of potato plants with mosaic to be 920 sq.cm., whereas that of the single normal plant measured was 2000 sq.cm. Though daily observations were made, the course of increase in total leaf area through the season is not presented. An estimate of the effect of virus infection on net assimilation rate is possible from Stone's data; using the daily leaf-area measurements and the final carbon content of the plants (apparently without allowing for the initial carbon content of the seed piece), he computed the amount of carbon accumulated/100 sq.cm./day; this was 37·3 mg. in the normal plant and 31·0 mg. in the infected plants. Taking the ash content into account (though again making no allowance for the initial dry weight, since this is not stated), these would correspond with net assimilation rates of 0·692 and 0·576 g./sq.dm./week, if a method of computation is used similar to that adopted previously for cacao (Goodall, 1950). This difference is considerably less than that found with virus 1A of cacao in the present experiments.

By gaseous exchange measurements, Thung (1928) found that leaf-roll of potato restricted photosynthesis, and Barton-Wright & M'Bain (1932) considered that their carbohydrate analyses confirmed this. It need not be supposed that these effects of viruses on photosynthetic efficiency are in general due to reduction in chlorophyll content, for Gabrielsen (1948) has shown that photosynthesis is little affected by chlorophyll content except under conditions of low light intensity where the chlorophyll content is considerably below normal; however, since the cacao seedlings of the present study were grown in shaded insectaries, it is possible that the reduced chlorophyll content might have played some part here.

Water content in the experiments of Ainsworth & Selman (1936) was depressed in

the early stages of infection, but later tended to be higher in the stems and roots of infected plants than in the controls. Caldwell (1934), on the other hand, found that infection with aucuba mosaic increased the water content of tomato leaves from 87·1 to 92·2% of the fresh weight. The scanty data of Stone (1936) also indicate a higher water content in the infected potato plants. It is possible that the cacao seedlings of the present experiments might at a later stage have shown, like the tomato plants of Ainsworth & Selman, a reversal of the observed reduction in water content, but over the period covered the effect seemed to be increasing with time from infection. The necrosis of fine roots may have been responsible for the low water content of the infected seedlings.

The observation on retarded loss of dry weight from the cotyledons is parallel with suggestions of interference with translocation in several other virus diseases. This is particularly noteworthy in potato leaf-roll and sugar-beet curly-top, where phloem necrosis is among the symptoms, but has also been reported in others such as potato paracrinkle (Barton-Wright & M'Bain, 1933).

In the present experiments as soon as visible foliar symptoms appeared the net assimilation rate was reduced. At the same time the proportion of plant dry matter represented by the leaves began to decrease, and the leaf-area ratio declined so that the relative growth rate was affected proportionately more than the net assimilation rate. At a slightly later stage, the proportionate development of lateral roots was also affected, and many of the fine roots suffered necrosis. While the proportion of new dry matter incorporated into leaf tissue and lateral roots decreased, that forming stem and tap-root tissue increased, suggesting that the virus had less adverse effect on protoplasmic synthesis (or reserve storage) in the stem and tap root than in leaves and fine roots; the characteristic symptom of swellings indicates that in some circumstances it may even stimulate cambial activity in the stem and root. At the same time as the virus is reducing the growth rate and affecting the proportions of the seedling, there is some evidence that it is also retarding the translocation of reserves from the cotyledons and hastening their abscission.

Infection also reduces the percentage water content of all parts of the plant, particularly the stem. Mature trees in the advanced stage of 'swollen-shoot' disease give the visual impression of dying from drought. This caused some confusion before the disease was shown to be caused by a virus, and 'drought die-back' and 'swollen-shoot' were associated in early reports (Dade, 1937). This similarity of 'swollen-shoot' to drought effects agrees with the observed reduction in water content, and may well be explained by the effects of the virus on root development. The fact that the root system is adversely affected (whether this is a direct effect or not) helps to explain why cacao trees are killed so rapidly by a virus which causes comparatively mild foliar symptoms.

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STUDIES ON POTATO VIRUS *X*

I. TYPES OF CHANGE IN POTATO VIRUS *X* INFECTIONS

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(With Plate 10 and 1 Text-figure)

In tobacco plants infected with mild strains of virus *X*, severe strains may arise as mutations which multiply locally.

Several strains of virus *X* gradually lost infectivity for potato on continued culture in other hosts such as tobacco.

Strains that are poorly infective or invasive for potato may take considerable time to move into the growing shoot from the tuber in detectable amounts.

Pronounced and apparently spontaneous increases in the severity of the strain that dominates in potatoes may occur in field crops.

Since Salaman (1938) described six strains of potato virus *X* it has become increasingly clear that there are many strains of this virus. The manner in which these strains arise and the way they change are questions of considerable biological and practical interest. Experiments are described which indicate that there may be several types of change.

The origin of strain mixtures in tobacco

Inoculations were made to *Nicotiana tabacum* (var. White Burley) from a fairly large selection of virus-*X* infected potato plants of standard English varieties. Most of these gave rise, in tobacco, to a mild mottle with a variable number of necrotic etched or yellowish spots. From these spots by leaf dissection severe-type strains could be isolated. As a preliminary to comparative study, attempts were made to obtain such mild and severe isolates in single-strain culture. This was done by isolation from single lesions produced with highly diluted inoculum.

A preliminary experiment using mixtures of two symptomatologically distinct and apparently pure and stable strains of virus *X* showed that each strain could be recovered alone from systemically infected tobacco plants inoculated with a mixture of the two strains at a sap dilution of 10^{-5} or 10^{-6}.

Preliminary tests of this technique with the naturally occurring mild strain mixtures showed that repeated isolation from single local lesions at high dilution did not free the mottle type strains completely from severe strains, in spite of the fact that the former must have been present in much greater amount than the latter.

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A further experiment was made to test the effect of dilution on the numbers and type of local lesions and systemic spots, using such naturally occurring mixtures of strains.

Five different sources of virus were used, all of the same general type as judged by symptoms on tobacco under glass. They all gave some type of mottle with scattered or yellowish systemic spots, i.e. they were all strain mixtures. Previously, extensive 'leaf dissection' had been carried out on these cultures, and all originally came from a green-mottled area of leaf showing no etched or yellow spots. The type of local lesion produced by these mixtures varied considerably with the environment. During winter the local lesions were mainly necrotic. During summer the mottle strains gave no chlorotic-type local lesions, and only scattered necrotic or ringspot local lesions due to severe strains appeared. During spring and autumn both types of strain gave distinct local lesions, the mottle strains giving chlorotic local lesions, the more severe strains giving ringspot or necrotic local lesions.

These local-lesion types had been tested previously and a rough separation of strains obtained. The experiment was done in April so that the local lesions of both types of strain could be detected and distinguished. Sap from infected tobacco was diluted 10^{0} to 10^{-6} with water, and inoculated at each dilution to three tobacco plants (using fine carborundum as an abrasive). The local lesions were counted 8 days after inoculation. When systemic symptoms were well developed (3 weeks after inoculation) the etched or yellow systemic spots were counted. The results are summarized in Table 1. Text-fig. 1 shows the average trend of the number of local lesions and systemic spots with increasing dilution for all sources combined.

Nine days after inoculation twelve chlorotic and four necrotic well-spaced local lesions were cut from plants inoculated with highly diluted sap; each was inoculated to one tobacco plant. Of the nine plants which developed mottle-type symptoms, six also developed some etched and necrotic systemic spots. Among some of these isolates, on further transfer, different mottle types could be distinguished originating from the same source.

A number of such isolates has been maintained for 2 years in tobacco or *N. glutinosa*. Most derived from chlorotic-type local lesions developed necrotic spots in the first transfer. Those which did not in the first did so in subsequent transfers. However, of five isolates causing ringspot symptoms, none showed any detectable mixture with mottle-type strains over the 2-year period.

In three of five plants inoculated at high dilution, systemic necrotic spots developed, although no necrotic local lesions had been found. Systemic spots also arose regularly in plants inoculated from single chlorotic local lesions isolated from leaves inoculated at a dilution of 10^{-5} or 10^{-6}.

Several mottle types were isolated from a single mottle source, and these mottle types remained distinct through successive transfers. This indicated that such isolated local lesions contained one strain only.

It is concluded that the systemic spots are produced both from virus that entered in the inoculum and from newly mutated particles.

In Text-fig. 1 (the average result for all sources) the number of systemic spots decreases with dilution to about 10^{-3} and then remains fairly constant. The fall between 10^{0} and 10^{-3} probably represents the effect of dilution on the initial mixed

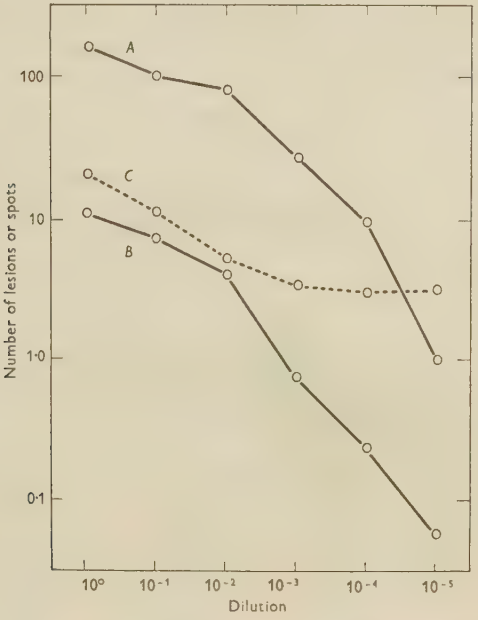
TABLE 1. *Effect of dilution on local lesions and systemic spotting*

Virus source	Dilution	No. of local lesions (mean of 3 leaves)		No. of systemic yellow or etched spots (mean of 6 leaves)
		Chlorotic	Necrotic or ringspot	
Ballard *X*	10^{0}	170	7	2·16
	10^{-1}	140	6	2
	10^{-2}	147	9	4
	10^{-3}	38	0·3	3
	10^{-4}	11	0·3	0·83
	10^{-5}	2	0	No systemic symptoms
	10^{-6}	2·3	0	No systemic symptoms
Arran Viking	10^{0}	183	6·6	1·5
	10^{-1}	134	3·3	3·8
	10^{-2}	20·5	0·5	3·7
	10^{-3}	12	0·3	1·3
	10^{-4}	0·6	0	1·8
	10^{-5}	0·3	0	1·5
	10^{-6}	0	0	No systemic symptoms
X^{L} (Salaman's stock)	10^{0}	150	13	2·8
	10^{-1}	51	6·3	1
	10^{-2}	52·3	3·3	0·83
	10^{-3}	42·3	1·3	1·3
	10^{-4}	27·3	0·66	1·66
	10^{-5}	2·3	0·3	1·66
	10^{-6}	0	0	No systemic symptoms
W. 3	10^{0}	98·3	22·3	77
	10^{-1}	96·6	18	41
	10^{-2}	80	5·66	16
	10^{-3}	26·6	1·66	10·2
	10^{-4}	4·3	0·3	3
	10^{-5}	0	0	2·83
	10^{-6}	0	0	1·5 (mean of 4 leaves)
Dakota	10^{0}	210	4	16·8
	10^{-1}	106·6	2·3	8·66
	10^{-2}	105	2	5·2
	10^{-3}	16	0·3	1·66
	10^{-4}	4·66	0	8·2
	10^{-5}	1	0	6·75 (mean of 4 leaves)
	10^{-6}	0·66	0	5 (mean of 2 leaves)

inoculum. The fluctuating number of spots between 10^{-3} and 10^{-5} may represent those spots that occur because of new mutations and therefore independent of dilution. The fact that the numbers of chlorotic and necrotic local lesions fall off

proportionally at the lower dilutions suggests that most necrotic local lesions do not contain fresh mutations, but strains derived from the inoculum.

Single-lesion cultures of mottle strains soon gave rise to severe strains, whereas severe strains remained apparently free from mottle-type strains, suggesting that the parent strains were of the mottle type with a fairly high forward mutation rate to severe types, and a much slower back mutation rate severe → mild. However, among the mottle-type isolates examined, T.B.R. *X* (a strain from tomato (Smith, 1946)) and *B* (from Duke of York (Bawden & Sheffield, 1944)) were exceptional in



Text-fig. 1. Effect of increasing dilution on the numbers of local lesions and systemic spots. *A* = chlorotic local lesions; *B* = necrotic or ringspot local lesions; *C* = systemic spots.

that, over a period of 2 years, they showed no evidence of having given rise to any severe strains. Thus it seems probable that there are wide differences in the mutability of different strains.

The reaction of *Cyphomandra betacea* to strain mixtures

Experiments on this problem have already been described (Matthews, 1949). The symptoms produced by potato virus *X* in this plant are variable. Pl. 10, figs. 1 and 2, show two types of mottle; fig. 3 shows a tobacco leaf inoculated with a source of potato virus *X* before passage through *Cyphomandra betacea*; fig. 4 shows the

increased severity of the strain after passage through *C. betacea*. It was concluded (Matthews, 1949) that *C. betacea* selects out severe strains at the expense of mild ones from a pre-existing mixture containing predominantly mild strains.

Loss of infectivity for potato on culture in non-potato hosts

In an experiment made in 1946 on the effect of virus *X* on the yield of potatoes, three different sources of the virus were inoculated in the field to three varieties of potato. The results of the tests to determine the number of plants infected are summarized in Table 2. K.P. *X* was a mild strain mixture from Kerr's Pink stock seed. A.P. *X* was a ringspot type strain from Arran Peak. T.B.R. *X* was a mottle type strain from tomato.

Table 2. *Infections obtained with virus X in field tests*

X strain used	No. of plants tested	No. of plants infected		
		Gladstone	Great Scot	Kerr's Pink
Water-inoculated control	16	0	1	1
K.P.	16	16	16	16
T.B.R.	16	11	11	3
A.P.	26	5	1	15

The tests on plants inoculated with A.P. *X* were carried out in three lots. Table 3 gives the details for this strain.

Table 3. *Plants infected with A.P. X*

	Test between 3. vii and 21. vii	Test of 13. viii.	Test of 20. viii.	Infected total out of 26 plants
Kerr's Pink	5/8	4/8	6/10	15
Gladstone	4/8	0/8	1/10	5
Great Scot	1/8	0/8	0/10	1

The difference in numbers of infected plants obtained between Kerr's Pink and Great Scot is significant at 0·05 *P*. These results suggested that there may be differences in the infectivity of various strains for the different varieties, and in 1947 a further trial was made to test this point.

Seed-size tubers of Gladstone, Great Scot and Kerr's Pink from the control plots of a yield trial carried out in 1946 were used. These potato stocks had been grown for 3 years at Cambridge, and some plants were known to be infected with potato virus *Y* and leaf-roll, but only a few with virus *X*. Planting was carried out in early May. Before inoculation they were tested for the presence of *X* by the group-testing method of Markham, Matthews & Smith (1948). The results indicated a very low infection with *X* virus.

The strains and sources of virus used were:

(1) K.P. *X*—a strain mixture carried symptomlessly by Kerr's Pink stock seed and bulked in tobacco.

(2) T.B.R. *X*—bulked in tobacco for inoculum.

(3) A.P. *X*—bulked in tobacco for inoculum.

(4) A.P. *X* A.V.—the same strain as in (3) from a potato source of inoculum (Arran Victory) (transmitted to Arran Victory by inoculation in 1946). Sources (3) and (4) were included to give a direct comparison of the effect of the source of inoculum on infectivity.

(5) 'Severe *X*'—this source was obtained from Clinch in 1946 in Arran Consul and was transmitted to Arran Victory by grafting in 1946. A plant grown from the grafted Arran Victory was used as a source of inoculum (conspicuous mottle but no necrosis on this plant in the glasshouse—giving on *Datura tatula*, etc., fairly mild symptoms).

For inoculations in the field the plants were minced, the sap expressed, and diluted 1 in 2 with water. A pad of muslin and cotton-wool, sprinkled with fine carborundum, was used to inoculate by light rubbing at least one leaf on each stem of each plant. Each strain source was inoculated to thirty plants of each variety on 20 June when the plants were 6–8 in. high. The arrangements of the treatments are shown in Table 4, the numbers (1)–(5) corresponding to the five strain sources.

TABLE 4. *Field infections with virus X. Numbers of infections obtained in groups of ten plants*

		No. of plants infected		
Virus inoculated	Plants tested	Gladstone	Great Scot	Kerr's Pink
1. K.P. *X*	1–10	8	6	9
ex tobacco	11–20	5	5	9
	21–30	6	8	9
2. T.B.R. *X*	1–10	0	0	0
ex tobacco	11–20	0	0	0
	21–30	0	0	0
3. A.P. *X*	1–10	0	0	1?
ex tobacco	11–20	0	0	0
	21–30	0	0	0
4. A.P. *X*	1–10	2	8	2
ex Arran Victory	11–20	3	3	3
	21–30	6	4	5 (+1 mild mottle)
5. Severe *X*	1–10	9	8	10
ex Arran Consul	11–20	9	8	8
	21–30	9	8	9
Control plots	1–10	0	0	0
	11–20	0	1	0
	21–30	0	0	0

The relative concentrations of virus in the inocula (except K.P. *X*) as used in the

field were estimated serologically by the β optimal proportions method, using an antiserum prepared against the severe *X* source. The results indicated no wide differences in concentration except that T.B.R. *X* was about one-quarter to one-eighth the concentration of the other strains.

Each field inoculum was tested on three tobacco, three *Datura tatula* and two *Nicotiana glutinosa* plants, all of which developed symptoms typical of the virus source involved.

During the season no symptoms could be noted on any plants. However, a dry summer made the detection of *X* symptoms difficult. The plants were tested between 11 and 20 August, when a single leaflet was taken from a young leaf of each stem of each plant. Sap from the bulked leaflets from each plant was inoculated to two tobacco and two *Datura tatula* plants.

Table 5 is a summary of all the information available on infectivity of three strains for potato. In this table the percentages of infection are for the three potato varieties taken together. It was not possible to determine exactly how many times A.P. *X* and T.B.R. *X* had been transferred in tobacco and *Nicotiana glutinosa* before they were obtained by the writer, but Dr K. M. Smith supplied an estimate. However, there is no definite evidence that T.B.R. *X* was ever in potato. In Table 5 the number of transfers is counted from the time T.B.R. *X* was reported in tomato.

Table 5. *Infectivity of different strains of virus X for potato*

Strain of virus	Source of virus for inoculation	No. of previous transfers in non-potato hosts	Time in months in non-potato hosts	Percentage infection in potato
A.P. *X*	Potato (1947)	0	0	40
A.P. *X*	Tobacco (1946)	14	About 18	27
A.P. *X*	Tobacco (1947)	20	About 30	0
T.B.R. *X*	Tobacco (1946)	14	About 18	52
T.B.R. *X*	Tobacco (1947)	19	About 30	0
K.P. *X*	Potato (1946)	0	0	100
K.P. *X*	Tobacco (1947)	1	1	72

Test of second year infection with A.P. *X* and T.B.R. *X*

On 14 April 1947 tubers from plants of the 1946 yield trial known to be infected with A.P. *X* or T.B.R. *X* were planted in the field. On 9 June the plants were small and showed no symptoms. They were tested by inoculation to two tobacco seedlings. On 4 July symptoms in the field were noted. On 21 August the plants were retested by inoculation to four tobacco plants each. Sampling for all tests was made by taking a single leaflet of each shoot of a plant. Some Gladstone and Great Scot plants infected with A.P. *X* showed a mottle in some shoots only. Table 6 summarizes the results.

In the first test to tobacco, when the potato plants showed no mottle symptoms, only a few infections were obtained. However, when some plants developed mottling

(before the plants were in leaf contact) it was seen that more plants were infected than the first test showed, and this was confirmed by the second test.

TABLE 6. *Numbers of virus-X infected plants from infected tubers*

Strain of virus	Variety of potato	No. of plants tested	No. of plants giving infections on tobacco on 9. vi. 47	No. of plants showing virus *X* mottle on 14. vii. 47	No. of plants giving infections on tobacco on 21. viii. 47
A.P. *X*	Kerr's Pink	19	7	13	13
T.B.R. *X*	Kerr's Pink	16	0	0	2
A.P. *X*	Gladstone	17	0	7	9
T.B.R. *X*	Gladstone	15	2	0	11
T.B.R. *X*	Great Scot	17	3	0	4

CORRELATION BETWEEN PERCENTAGE INFECTION IN PLANTS AND TUBERS

The figures for the number of plants infected by inoculation in 1946 and the number of infected tubers from infected plants (tested in 1947) are given in Table 7. The correlation coefficient = 0·82 is significant at the 0·05 *P* but not 0·02 *P*. Such a result would be expected on the basis of a correlation between infectivity and invasiveness. On the other hand, such a correlation would appear if all the tubers from each infected shoot became infected, but the percentage of shoots infected (in infected plants) was correlated with the percentage of plants infected.

TABLE 7. *Infection in plants and tubers*

		Plants infected following inoculation in 1946			Infected tubers from infected plants (tested in 1947)		
Variety of potato	Strain of virus	No. of plants tested	No. of plants infected	% infected	No. of tubers tested	No. of tubers infected	% infected
Gladstone	K.P. *X*	16	16	100	15	14	94
Great Scot	K.P. *X*	16	16	100	20	18	90
Kerr's Pink	A.P. *X*	26	15	58	19	13	68
Kerr's Pink	T.B.R. *X*	16	3	19	16	2	13
Gladstone	A.P. *X*	26	5	19	17	9	53
Gladstone	T.B.R. *X*	16	11	69	15	11	73
Great Scot	T.B.R. *X*	16	11	69	17	4	24

The data of Table 5 strongly indicate that the strains of virus *X* used progressively lost infectivity for potato after culture in non-potato hosts (chiefly tobacco). In view of this it is difficult to make any statement about the relative infectivities of the three strains had they all been cultured continuously in potato. One explanation could be that the plants were not uniform in susceptibility, but this is improbable. Likewise, the idea that some constituent of tobacco sap might inhibit infectivity would not explain the decrease in infectivity in successive seasons. The number of possible entry points made available by the technique used was far in excess of the number required to give infection. If isolates lose infectivity for potato on passage through tobacco it may be that, in some of the inocula that infected less than 100% of the

inoculated plants, the few virus particles present were infectious to potato. It could be imagined that a strain transferred from potato to tobacco, although multiplying in the latter, would not be as well adapted to it as mutants appearing from time to time. These would then gradually replace the original by a process of natural selection. Such adaptation might not be accompanied by any other obvious changes. In this connexion, A.P. *X* from infected plants in the field* (1947) was compared in parallel inoculations on tobacco, *Nicotiana glutinosa* and *Datura tatula* with A.P. *X* from stock tobacco plants. No differences in the time of appearance or nature of symptoms could be detected.

Further experiments will be necessary to determine whether number of transfers or merely time is of importance in the process leading to loss of infectivity. Adaptation or change would probably be more likely to occur during multiplication and spread of the virus in the plant.

It is clear from the results of 1947 tests on the progeny of plants infected with T.B.R. *X* and A.P. *X* in 1946 that the virus may take some time to move up into the growing shoots from the tuber in detectable amounts. This casts doubt on the efficiency of those methods of testing for virus *X* which involve inoculations from sprouting eyes or young shoots. However, there is fairly good evidence that these strains, as used, were not very infective or invasive. In the usual type of mild infection found in the field, the movement of virus from the tuber may occur earlier, and the rate of spread and multiplication in the shoots may well be greater.

Changes in type of virus *X* infection in potatoes in the field

Clinch (1944) described a severe strain of potato virus *X*, found occurring naturally in seed-potato stocks, which underwent a striking decrease in virulence over a period of several seasons. In this section some observations are described which show that mild strains may become more severe.

In 1946, 160 plants of Great Scot were inoculated in the field with K.P. *X*, a mixture of strains from stock seed Kerr's Pink containing mainly mild with a few severe strains. Only one plant developed symptoms, which appeared on one shoot as systemic necrotic rings and spots. Inoculations from all the shoots of this plant to tobacco and *Datura tatula* showed that the symptomless shoots contained the mild K.P. *X* mixture approximately as inoculated, while the necrotic shoot appeared to contain only a very severe strain.

As part of an experiment carried out in collaboration with Mr J. C. Cullen of the National Institute of Agricultural Botany, a large number of plants of virus-free Kerr's Pink and Arran Banner were inoculated, in the field, in Ulster in July 1947 with sources of virus *X* obtained from symptomless Scotch stock seed Kerr's Pink and Arran Banner respectively. These two sources gave on tobacco a mild mottle

* One of these plants (Great Scot) was found to be infected with Spotted Wilt virus. Infection must have taken place during the current season, possibly from dahlia plants growing nearby.

with a few scattered etched and necrotic or yellow flecks, i.e. they were mixtures of mainly mild with a little severe strain.

In 1947 the inoculated potatoes showed no symptoms. Tubers from this crop were planted in Ulster in 1948. On inspection in July it was found that 10–15% of the Kerr's Pink, and less of the Arran Banner plants, showed conspicuous mottling, necrotic spotting, or severe necrosis in some shoots, while other shoots remained symptomless. Shoots from three such plants were tested by inoculation to tobacco and *D. tatula*. The symptoms on these hosts were correlated with those on the potato shoots. Symptomless shoots contained mixtures similar to the original type used for inoculation; mottled shoots contained more severe strains, while necrotic shoots produced in tobacco and *D. tatula* severe ringspot and a necrotic type of *X* infection.

In 1944 the variety Arran Viking at Arran obtained an S.S. certificate, although there are good reasons for believing that it was already extensively infected with virus *X*. In 1945 30% of the plants of the stock were mottled. Selection of mottle-free plants from this stock gave a definite raising of the visible standard in 1946 (H → A certificate, compared with unselected stock with 30% mottle). From serological and inoculation tests the total *X* infection in Arran Viking at Arran in 1946 was estimated at about 75%. As judged by symptoms on *D. tatula*, mottled plants contained more severe type strain than did symptomless plants. Thus there is some circumstantial evidence from the history of this variety that the type of virus *X* infection was tending to become more severe.

Whether these more severe types of infection arise by very recent mutation from the mild types, or whether they had been present in small quantities in the inoculum, or in the potato stock, it seems clear that marked, and apparently spontaneous increases in the severity of the dominant strain in virus *X* infection may occur in potatoes in the field.

Discussion

Experiments have been described which indicate that potato virus *X* infections may change in the following ways:

(i) Mutation.

(ii) Selection of one type of strain from a mixture by a particular host plant.

(iii) Gradual loss of infectivity for potato on continued culture in non-potato hosts.

(iv) Sudden spontaneous changes in the dominant strain type in all or part of a potato plant.

If viruses do not arise *de novo*, then new strains must arise from pre-existing ones by a process akin to mutation. Although it is difficult, if not impossible at present, to prove that a mutation has occurred in any particular case, evidence has been presented which indicates that mutations frequently do occur and that the commonly found mixed virus *X* infections arise, at least in part, in this way.

It is probable that many different types of mutants are produced. Only a superficial examination has been made of a few strains and of some of the strains which apparently arise by mutation from them. In the material examined it seems likely that the parent strains are of the mottle type and that the common mutations are to the ringspot or severe types. There are, however, two respects in which these conclusions may be limited. First, the material examined was largely from potato stocks in which artificial selection for symptomless strain types had been practised; in strain populations, where severe types are dominant, the types of mutant commonly appearing may be quite different. Secondly, a more serious limitation is that we can detect only those mutants which give a visibly different reaction in the host. The apparent predominance of severe-type mutants may well be due to the fact that only this type of mutant can be detected and isolated in a mottle-infected tobacco plant. Because of this inability to detect all types of mutants, no reliable estimate of the rate of mutation can be made. The data in Table 1 on the number of systemic spots arising from infection at high dilution would probably give a rough estimate of the minimum number of mutants per plant for these particular strains. The data indicate a minimal number of mutants of about three per leaf.

There is at present no evidence for any other type of change in the virus particle other than a stepwise change akin to mutation. Mutation probably provides the basis for change in the other phenomena described. It has been suggested (Matthews, 1949) that *Cyphomandra betacea* selects out severe-type strains from a pre-existing mixture of mild and severe. Presumably such mixtures arose by mutation at some previous time.

The explanation of the gradual loss of infectivity of virus *X* for potato on culture in tobacco is at present obscure. Virus *X* infections may suddenly become either more or less severe in potatoes. No doubt mutation provides the source of variability. Further work will be necessary to determine whether the dominant strain changes because a mutant more invasive than the parent has arisen, or whether it is merely a matter of chance, among the various strains present, which happens to have become established first in a particular growing shoot.

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Fig. 1

Fig. 2

Fig. 3

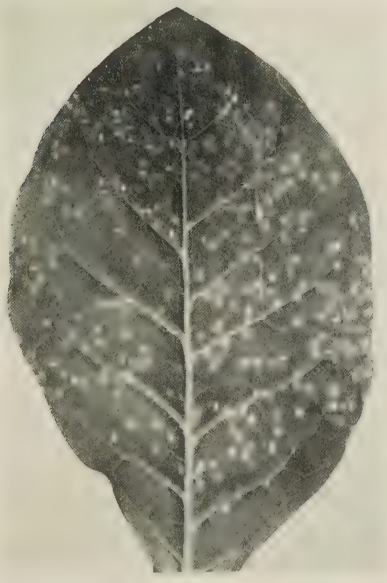

Fig. 4

EXPLANATION OF PLATE 10

Fig. 1. *Cyphomandra betacea* inoculated from Dakota Red potato. Fine systemic mottling.

Fig. 2. *Cyphomandra betacea* inoculated from Dakota Red potato. Coarse, blotchy, interveinal yellowing.

Fig. 3. Tobacco inoculated from Dakota Red potato. Chlorotic-type local lesions.

Fig. 4. Tobacco inoculated from *Cyphomandra betacea*. Ringspot local lesions. Cf. fig. 3.

STUDIES ON POTATO VIRUS *X*

II. CRITERIA OF RELATIONSHIPS BETWEEN STRAINS

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(With Plate 11)

The range and type of symptoms produced by strains of virus *X* are briefly described.

Of ten strains examined, six were identical in serological cross-absorption tests, and two (T.B.R. *X* and *B*) differed considerably from the others. The possibility of preparing strain-specific antisera is indicated.

The protection afforded by mild strains against severe strains in tobacco and *Datura tatula* was complete for all strains tested except T.B.R. *X* and *B*, which gave only partial protection. Thus there is a correlation between wide serological differences and a breakdown in the protection afforded in the plant.

There was no correlation between type of symptoms caused and serological relationships.

There was some evidence to show that strains from the same source were more closely related than strains from different sources.

The main basis for the description and classification of strains of potato virus *X* has been on symptoms produced on some selected or conventional range of host plants. Thus Salaman (1938) attempted to identify all the strains described in the literature with the six types he had isolated, largely on a basis of symptoms in some solanaceous hosts. However, it has long been recognized (e.g. Bawden & Pirie, 1937) that symptoms alone are unsatisfactory as a basis for differentiating and grouping viruses.

In the present work an attempt has been made to evaluate some of the various lines of evidence at present available for establishing relationships between strains of potato virus *X*.

The symptoms produced by strains of potato virus *X*

The most widely used grouping of strains is the division into 'mottle' and 'ringspot' groups as shown by symptoms on tobacco and other non-potato hosts. Although these are useful descriptive terms, such a separation may have no firm basis. The history of work on potato virus *B* well illustrates the danger of using symptoms as a basis for identification.

In 1936 Bawden described a virus in Up-to-Date potatoes which he called virus *B*, distinguished from virus *X*, with which it was mixed in Up-to-Date, partly on account of its reactions on some potato varieties. Salaman (1938) did not recognize *B* as a strain of virus *X*. It was later shown to be so by Clinch (1942), but was

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considered to be a rather aberrant type. In 1943, Cockerham described a virus in Duke of York which he considered to be pure virus *B* as originally described by Bawden in Up-to-Date. He identified his virus with Bawden's chiefly on account of its reactions on certain potato varieties. Bawden & Sheffield (1944), as the result of their work on virus *B* from Duke of York and on the X^a strain, pointed out that a simple test based on host reactions is not a valid test of relationship. The streak virus in Up-to-Date and *B* in Duke of York have been found (Matthews, 1947) to differ serologically, and in the present work further differences have been found.

The range of symptoms produced by different strains

On the three species mainly used in this work, tobacco (White Burley), *Nicotiana glutinosa* and *Datura tatula* (the purple-flowered variant of *D. stramonium*) strains of virus *X* have been found which, under the same environmental conditions, can produce widely differing symptom pictures, ranging from strains which are carried without symptoms to those which cause severe necrosis. Between these extremes a wide variety of symptoms may develop: Various types of fine or coarse, diffuse or well-marked green banding of the veins; faint yellow-green, or bright yellowish, irregular mottles; faint yellow spotting; yellow-etched spotting; etched ring and line patterns; necrosis spreading along the veins. If local lesions develop, they may be very faint, diffuse, pale spots; well-marked whitish or yellow spots; well-marked etched ringspots; necrotic ringspots; or solid necrotic local lesions.

The effect of environmental conditions on symptoms

It is well known that the conditions under which the plant is grown may greatly affect the symptoms produced by a virus. No systematic study has yet been made of the effect of environmental conditions for different virus strain-host plant combinations. However, the results of some preliminary experiments, and observations made under varying seasonal conditions showed that:

(1) Changes in light and temperature can cause almost as much variation in symptoms as that caused by different strains.

(2) Strains which are obviously different under one set of conditions may appear identical under different conditions.

(3) In an experiment in which a uniform set of tobacco plants was inoculated with a ringspot of potato virus *X* and batches of these plants placed immediately after inoculation under three different light intensities (60, 130 and 260 foot-candles at plant surface, tungsten bulb source), it was found that the ratios of the concentration of the virus in the sap of these plants 8 days after inoculation was about 1:16:2, i.e. with increasing light intensity the virus content at first increased and then decreased; this effect was immediate. In Bawden & Roberts's (1947) experiments the plants were kept under different light intensities for about 4 weeks before inoculation.

(4) The alternation of light and darkness plays no part in the formation of ringspots in potato virus *X* infections. Ringspot local lesions of the same type appeared simultaneously on tobacco plants continuously illuminated and on those that were illuminated with light of the same intensity and type for each alternate period of 12 hr. Air humidity and temperature were maintained at a constant level during the experiment.

The correlation between severity of symptoms in different host plants

Bald & White (1942) used the severity of symptoms in *D. stramonium* as an index of the severity of the strain mixtures in potatoes. With Clinch's (1944) severe *X* the symptoms in potato and in other solanaceous hosts are also correlated. The form severe for potato caused ringspot and necrotic symptoms in tobacco, *Nicotiana glutinosa*, *Datura*, etc., whereas the form from 'recovered' potatoes produced mild mottle-type symptoms on these hosts.

Correlation between symptoms produced on tobacco, *Nicotiana glutinosa* and *Datura tatula* by various strains is fairly close. Some evidence has been obtained that there may be a correlation between symptoms in *D. tatula* and in potato. For instance, in an investigation of the strains of virus *X* occurring in the variety Arran Viking, isolates from mottled potato plants were more necrotic on *D. tatula* than were isolates from symptomless plants. Similarly, a highly necrotic shoot on a potato found in the field yielded a type of virus which gave highly necrotic symptoms on tobacco. These facts indicate that there is frequently a correlation between severity of symptoms in different hosts. That this is not always so was pointed out by Bawden (1943). It is not possible to predict from the symptoms in *D. tatula*, tobacco, etc., what the reaction of a particular strain will be on any potato variety, as mild strains (defined on tobacco) may cause top-necrosis in a potato variety while a ringspot strain may give only a mild mottle in the same variety.

Symptoms produced by the isolates used for further study

The description given in Table 1 is intended to indicate the main similarities and differences in symptoms caused by the various strains.

In isolation experiments using the dissection and dilution technique many isolates were obtained, of which those described below are representative. The symptoms described are those produced under spring or autumn glasshouse conditions. In midsummer, symptoms tend to be masked, and in winter more severe. Table 1 summarizes the symptoms produced on tobacco (White Burley), *Datura tatula* and *Nicotiana glutinosa*. Tobacco and *Datura tatula* are better than *Nicotiana glutinosa* for showing small differences between strains. In the Table the strains are listed in approximate order of severity as judged by symptoms on these hosts.

The source of *B* virus in Duke of York potato obtained from Mr Bawden, and the source of Up-to-Date streak in Up-to-Date potato from Dr Clinch, were tested

by grafting directly to healthy plants of Epicure, King Edward, President and Arran Victory. The sources gave the reactions described in the literature, i.e. the Up-to-Date streak source gave top-necrosis in all four varieties; virus *B* in Duke of York gave top necrosis in President and Arran Victory, no symptoms on Epicure and King Edward. These two sources were inoculated directly to *N. glutinosa* for the tests described by Matthews (1947).

The isolates U.T.D. *M*, U.T.D. *R*, D.O.Y. *B*, and Dak. *M* were tested by inoculation and by grafting of *N. glutinosa* scions to two plants each of Epicure and President. These tests were made after the isolates had been cultured for over 12 months in tobacco and *N. glutinosa*. Top-necrosis developed in one of the Epicure plants grafted with U.T.D. *M* and one with U.T.D. *R*. No other plants developed symptoms. They were checked by inoculation to tobacco, which showed that D.O.Y. *B* had infected Epicure without causing symptoms. The other tests were negative. Presumably these isolates had lost infectivity for potato.

Serological relationships between some strains of potato virus *X*

Besides their use in the identification of a virus as a member of a group of strains, serological methods have been used to determine relationships between strains. Early workers attempted to do this by estimating the strength of the reactions of a strain with homologous and heterologous antisera. Chester (1936) first used the cross-absorption procedure with plant-virus strains, and worked out antigenic formulae for three strains of virus *X*: Potato Mottle *a*, *b*, *c*; Potato Ringspot *a*, *d*, *e*; and Masked Potato Mottle *a*, *b*, *d*.

Bawden & Sheffield (1944) determined the serological relationships between four strains of virus *X*. Their results suggested that the greater the difference in symptoms caused, the greater are the antigenic differences. Larson (1946) found in reciprocal precipitin tests that potato latent mottle, latent ringspot and virulent ringspot viruses were serologically indistinguishable. Matthews (1947) found by cross-absorption tests that the *B* virus in Duke of York was not serologically identical with the streak virus in Up-to-Date (the virus originally described as '*B*'). In view of the apparent discrepancies between the results of various workers, and as a part of the attempt to evaluate criteria for the identification of strains, cross-absorption experiments were made using a larger number of strains.

Strains of virus used

Ten strains of the virus were used. In view of the large number of cross-absorptions possible, the strains were examined in two groups and subsequent experiments were carried out linking the two groups together.

First group: X^L Mottle, X^L Ringspot, X^H Mottle, X^H Ringspot, Dakota Mottle, and Dakota Ringspot.

Second group: U.T.D. Mottle, U.T.D. Ringspot, T.B.R. *X*, and *B*.

TABLE 1. *Origins of and symptoms produced by strains of potato virus X*

Virus	Origin	Symptoms on *D. tatula*	Symptoms on tobacco (White Burley)	Symptoms on *N. glutinosa*
Dakota Ringspot	From a symptomless Red Dakota potato plant inoculated to tobacco. From tobacco a single local lesion isolation at high dilution	The most severe ringspot strain isolated—necrotic local lesions; systemic etching and much necrosis. Local lesions appear 24–48 hr. before any other strain	Ringspot local lesions; conspicuous systemic etched rings and line patterns	As for tobacco
U.T.D. Ringspot	Isolated by a similar process to Dakota Ringspot from an Up-to-Date plant supplied by Clinch	Necrotic local lesions and systemic etching and necrosis, less severe than Dak. *R*	Ringspot local lesions; systemic etched rings and lines	As for tobacco
A.P. *X*	Isolated by K. M. Smith from Arran Peak, and passed through single lesion culture in cucumber	Solid necrotic or ringspot local lesions; systemic etching and necrosis	Etched ringspot local lesions; systemic etched patterns	As for tobacco
X^H Ringspot	Isolated by a similar process to Dakota Ringspot from a President plant containing Salaman's X^H	As for A.P. *X* but slightly less severe	As for A.P. *X* but slightly less severe	As for A.P. *X* but slightly less severe
Larson's Ringspot *X*	From a seedling supplied by R. H. Larson	As for A.P. *X* but slightly less severe	As for A.P. *X* but slightly less severe	As for A.P. *X* but slightly less severe
X^L Ringspot	Isolated by a similar procedure to Dakota Ringspot from a President plant containing Salaman's X^L	Slightly less severe than X^H Ringspot	Slightly less severe than X^H Ringspot	Slightly less severe than X^H Ringspot
T.B.R. *X*	Isolated by K. M. Smith from a tomato fruit	Chlorotic local lesions; conspicuous irregular coarse yellowish mottle	Chlorotic local lesions; yellowish or whitish conspicuous irregular mottle often with etched patterns, especially in older leaves	Chlorotic local lesions; conspicuous fine vein yellowing; mottle less conspicuous than in tobacco
X^L Mottle	By the same procedure from the same source as X^L Ringspot	Chlorotic local lesions; systemic coarse irregular fairly conspicuous green-yellow-green mottle	Chlorotic local lesions; conspicuous irregular green-yellow-green mottle	As in tobacco but less conspicuous
Dakota Mottle	By the same procedure from the same source as Dakota Ringspot	Chlorotic local lesions; mild coarse irregular mottle with some green veinbanding	Chlorotic local lesions; mild irregular mottle	Faint irregular mottle
U.T.D. Mottle	By the same procedure from the same source as U.T.D. Ringspot	Chlorotic local lesions; mild mottle, irregular, and green veinbanding	Chlorotic local lesions; mild irregular green veinbanding mottle	Faint green veinbanding mottle
B	From a Duke of York plant supplied by F. C. Bawden	Faint chlorotic local lesions; mild, fairly even green veinbanding mottle	Faint chlorotic local lesions; mild fairly regular green veinbanding mottle	Faint green veinbanding mottle
X^H Mottle	By the same procedure from the same source as X^H Ringspot	Usually shows no symptoms. May give very faint green veinbanding	Usually shows no symptoms. May give very faint green veinbanding	Usually shows no symptoms

Preparation of virus

The various strains to be used were propagated in *Nicotiana glutinosa* in an attempt to avoid contamination with tobacco-mosaic virus. Plants were harvested 2–3 weeks after inoculation and frozen at −12° C.

No reliable method for the purification of virus *X* was available. The following procedures were used: Frozen leaves were ground or minced, thawed, and the sap expressed. In earlier preparations the sap was clarified by adding dipotassium phosphate followed by precipitation of the virus by one-third saturation with ammonium sulphate and resuspension of the virus in about one-fifth or one-tenth the original volume of water, followed by a further centrifugation to remove insoluble matter.

Some preparations made in this way contained less virus than the original sap, and with others almost all the virus became insoluble on standing for a few days or even hours. Such behaviour did not appear to be correlated with strain of virus or any other factor. Most of the later virus preparations were made simply by heat clarification (55° C. for 10 min.) of expressed sap from frozen leaves. Such preparations appeared to be stable at least over a period of a few days. However, not all of any particular strain was prepared at once, and when a sample became insoluble, more sap from the frozen material was clarified by heating. The only disadvantage in using such virus preparations was the limitation placed on the sensitivity of the cross-absorptions. It was usually necessary to add at least an equal volume of virus preparation for an antiserum to be fully absorbed.

Preparation of antisera in rabbits

Virus prepared as above (usually by the single ammonium sulphate precipitation method) was used for injection. A single intravenous injection of 1 ml. was given followed by bleeding after 2 weeks.

The antisera were tested for the presence of antibodies to tobacco mosaic over a range of tobacco-mosaic dilutions, with the antiserum diluted no more than 1 : 3. Of the ten antisera prepared, four reacted with tobacco-mosaic virus. Presumably the tobacco-mosaic virus came from a few undetected local lesions. Antibodies to this virus were removed by absorption with a suitable amount of a concentrated tobacco-mosaic virus solution. The antisera were also tested in a similar manner for reaction with healthy plant sap constituents. None of the ten antisera reacted with heat-clarified sap from healthy *N. glutinosa*. The titres of the various antisera with their homologous virus strains were determined (Table 2).

Cross-absorptions

Most antisera were fully absorbed with the addition of one or three parts of virus preparation at 1 : 1 to one part of antiserum at 1 : 1. If absorption was not complete after the first addition, further virus was added. The mixture for absorption was

kept at 53° C. for 2–4 hr., followed by 4–16 hr. standing at room temperature. The precipitate was then centrifuged off.

Tests after cross-absorption

In the tests for strain-specific antibodies after absorption, the homologous virus preparations were used as dilute as possible (two or four times the virus end-point concentration) to avoid inhibition of precipitation of a small amount of antibody by virus excess. Absorbed antiserum at a series of twofold dilutions from 1:1 to 1:32 was tested against homologous virus at a constant dilution.

The set of dilutions with a similar set of controls (absorbed antiserum with virus used for absorption) was then incubated at 53° C. for 2–6 hr. and any precipitates noted. The tubes were then set at room temperature overnight and the precipitates checked next morning.

TABLE 2. *Titres of antisera with homologous virus*

Strain of virus	Titre of antiserum	Strain of virus	Titre of antiserum
X^LM	1 : 16	Dak. *R*	1 : 512
X^LR	1 : 64	U.T.D. *M*	1 : 32
X^HM	1 : 64	U.T.D. *R*	1 : 16
X^HR	1 : 32	T.B.R. *X*	1 : 64
Dak. *M*	1 : 64	*B*	1 : 256

Results

Tables 3–6 summarize the results of the absorption experiments. The fractions indicate that strain-specific antibodies remained after absorption.

(*a*) *First series*

TABLE 3. *Reciprocal cross-absorptions*

Antiserum absorbed	Strain of virus used					
	X^LM	X^LR	X^HM	X^HR	Dak. *M*	Dak. *R*
X^LM	—	o	o	o	o	—
X^LR	o	—	o	o	—	o
X^HM	1/8	1/4	—	o	1/8	—
X^HR	1/2	1/2	o	—	—	1/2
Dak. *M*	o	—	o	—	—	o
Dak. *R*	—	o	—	o	o	—

Explanation of Tables 3, 4 and 6:

$$\text{The fraction} = \frac{\text{Titre of unabsorbed antiserum with homologous virus}}{\text{Titre of absorbed antiserum with homologous virus}}.$$

o = test carried out. No strain-specific antibody detected.

— = test not carried out.

(*b*) *Second series*

TABLE 4. *Reciprocal cross-absorptions*

Antiserum absorbed	Strain of virus used			
	U.T.D. *M*	U.T.D. *R*	*B*	T.B.R. *X*
U.T.D. *M*	—	o	1/2	1/4
U.T.D. *R*	o	—	1/8	o?
B	1/8	1/8	—	1/8
T.B.R. *X*	1/4	1/4	1/16	—

As a number of strain-specific fractions was found in the second series, further combinations of absorption and testing were possible. All the information obtained is summarized in Table 5. U.T.D. *R* was not used as it appeared to be identical with U.T.D. *M*.

TABLE 5. *Summary of absorption experiments between U.T.D.* M, B *and T.B.R.* X

Antiserum absorbed	Virus used for absorption	Virus used for test after absorption	Fraction titre after absorption / titre before absorption
U.T.D. *M*	*B*	U.T.D. *M*	1/2
U.T.D. *M*	T.B.R. *X*	U.T.D. *M*	1/4
U.T.D. *M*	*B*+T.B.R. *X*	U.T.D. *M*	≏1/10
U.T.D. *M*	T.B.R. *X*	*B*	1/4
U.T.D. *M*	*B*	T.B.R. *X*	0
B	U.T.D. *M*	*B*	1/8
B	T.B.R. *X*	*B*	1/8
B	U.T.D.M.+T.B.R. *X*	*B*	≏1/5
B	T.B.R. *X*	U.T.D. *M*	1/4
B	U.T.D. *M*	T.B.R. *X*	1/8
T.B.R. *X*	U.T.D. *M*	T.B.R. *X*	1/4
T.B.R. *X*	*B*	T.B.R. *X*	1/16
T.B.R. *X*	U.T.D. *M*+*B*	T.B.R. *X*	≏1/20
T.B.R. *X*	U.T.D. *M*	*B*	1/32
T.B.R. *X*	*B*	U.T.D. *M*	0

(*c*) *Experiments linking the first and second series*

An X^H specific antiserum was prepared by absorbing X^H Mottle antiserum with X^L Mottle virus. None of the four strains of the second series reacted with this antiserum.

Cross-absorptions between two pairs of strains are shown in Table 6.

TABLE 6. *Cross-absorptions*

Antiserum absorbed	Virus used for absorption: Dak. *M*	U.T.D. *M*	X^LM	*B*
Dak. *M*	—	0	—	—
U.T.D. *M*	0	—	—	—
X^LM	—	—	—	1/8
B	—	—	1/8	—

From the results of the above experiments the following formulae are assigned:

Dak. *M*, Dak. *R*, X^LM, X^LR, U.T.D. *M*, U.T.D. *R*: *G*, *a*, *b*

X^HM, X^HR: *G*, *a b*, *h*

B: *G*, *b*, *c*, *e*

T.B.R. *X*: *G*, *c*, *g*

Experiments on cross-protection between strains of virus *X*

Thung (1931) and Salaman (1933) showed that infection with one strain of a virus could protect a plant from infection with a second strain. The phenomenon has since come to be used as a criterion of relationship between viruses, although its basis is not clearly understood.

Various workers report different results with virus *X*. Smith (1933) described a case in which a severe type of *X* formed local lesions when inoculated into a tobacco infected with a mottle strain. Salaman (1938) does not mention this observation and considered that cross-protection was complete between all his strains. Sadasivan's (1940) work with X^S and X^G supported this view. Clinch (1942) found that the presence of virus *X* in potato plants protected them from infection by inoculation with the Up-to-Date streak virus. Bawden & Sheffield (1944) found virus *B* from Duke of York protected against other strains, although the protection was often not absolute, e.g. the virus *X* largely protected *Datura stramonium* from *B* but sufficient entered to cause top-necrosis when *Datura* scions were grafted to intolerant potato hosts. Smith (1946) described two strains—a mottle type from tomato (T.B.R. *X*) and a severe type from Arran Peak potato (A.P. *X*)—of which the former did not protect completely against infection with the latter. Because of these somewhat conflicting results systematic experiments using a larger number of strains were made.

Experiment in tobacco

For the first inoculation the 'Mottle' type strains used were: Dakota Mottle, X^L Mottle, X^H Mottle, U.T.D. Mottle, T.B.R. *X* and *B*. For the second inoculation the 'Ringspot' types used were: Dakota Ringspot, X^L Ringspot, X^H Ringspot, U.T.D. Ringspot, Larson's Ringspot and A.P. *X*. On 4 September 1947 each of the above mottle strains was inoculated to twenty-one young White Burley tobacco plants. Twenty-one uninoculated plants were kept as controls.

By 25 September systemic symptoms were well developed (except X^H Mottle). On 26 September each of the ringspot strains was inoculated to three plants of each of the mottle strains plus three healthy plants as controls, using fine carborundum as an abrasive. Three plants of each of the mottle strains were rubbed with water as controls.

On about 1 October local lesions appeared on all the healthy control plants inoculated with ringspot strains (the local lesions for Dakota Ringspot appeared 12–24 hr. before the others). At the same time similar but less numerous local lesions appeared on certain of the mottled plants. These results are summarized in Table 7. When local lesions of second strain developed on top of mottle of the first strain, the breakdown is recorded by a cross. In most other plants no local lesions or systemic symptoms of the second strain developed. However, in a few some local lesions developed, and by their distribution were not considered to indicate a real breakdown in cross-immunity; e.g. one or two small clusters of local lesions in

isolated areas on three plants, or one leaf covered with local lesions, all the others having none. These cases are marked by a ? in the Table. In none of these plants did systemic symptoms of the severe strain develop, while in a number of plants in which local lesions developed as in the controls, systemic spread of the second strain followed.

Concentration of virus. The concentration of the virus present for the various mottle strains was measured serologically using optimal proportions. Sap from control tobacco plants was tested on 18 October. The optimal proportions tube for all six strains was within a twofold dilution range.

TABLE 7. *Cross-immunity in tobacco*

		2nd strain (Ringspot type)					
1st strain (Mottle type)	Breakdown	Dak. Ringspot	X^L Ringspot	X^H Ringspot	U.T.D. Ringspot	Larson's *X*	A.P. *X*
Dak. Mottle	Local	–	?	–	–	–	–
	Systemic	–	–	–	–	–	–
X^L Mottle	Local	–	–	–	–	–	–
	Systemic	–	–	–	–	–	–
X^H Mottle	Local	?	–	?	–	?	–
	Systemic	–	–	–	–	–	–
U.T.D. Mottle	Local	–	–	–	–	–	–
	Systemic	–	–	–	–	–	–
T.B.R. *X*	Local	+	+	+	?	?	+
	Systemic	2/3 pls.	1/3 pls.	–	–	–	–
D.O.Y. *B*	Local	+	+	+	+	+	+
	Systemic	2/3 pls.	1/3 pls.	1/3 pls.	2/3 pls.	3/3 pls.	–

– = no local or systemic breakdown. + = breakdown occurred.

Experiment with Datura tatula

On 23 October seven pots of six *Datura tatula* plants were inoculated with each of the six mottle strains. On 13 November, when systemic symptoms were well developed (except X^H which showed none), six plants with each mottle strain were inoculated with each of the six ringspot isolates, using fine carborundum. Sets of control plants were used, as in the tobacco experiment.

Five days after inoculation, numerous small black necrotic local lesions appeared on the healthy plants inoculated with the Dakota ringspot strain. On the same day local lesions, not as numerous as in the controls, appeared on mottled leaves infected with the T.B.R. *X* and *B* strains but on none of the others. Two days later necrotic local lesions developed on the healthy plants inoculated with the other five severe strains, and well-marked but less numerous necrotic local lesions developed on: U.T.D. *R* on *B* and T.B.R. *X*, X^L on T.B.R. *X* and A.P. *X* on *B*. Similar local lesions developed on the other T.B.R. *X* and *B* plants a day or so after they appeared on the controls. No necrotic strain local lesions or systemic symptoms appeared on any of the other four mottle strains.

Subsequently the necrotic strains killed the healthy control *D. tatula* plants. In the T.B.R. *X* and *B* plants local lesions of the severe strains were not so numerous, but they did coalesce in places. Systemic necrosis, indicating systemic spread of the severe strains, began to appear in all the T.B.R. *X* and *B* plants about 3 weeks after inoculation with the severe strain. This necrosis did not affect the plants as in the controls.

The results of this experiment are summarized in Table 8 and illustrated in Pl. 11.

TABLE 8. *Cross-immunity between strains in* Datura tatula

Mottle strain	Ringspot strain					
	Dak. *R*	X^LR	X^HR	U.T.D. *R*	A.P. *X*	Larson's *X*
Dak. *M*	–	–	–	–	–	–
X^LM	–	–	–	–	–	–
X^HM	–	–	–	–	–	–
U.T.D. *M*	–	–	–	–	–	–
T.B.R. *X*	+	+	+	+	+	+
D.O.Y. *B*	+	+	+	+	+	+

– = no local or systemic breakdown. + = breakdown occurred.

On 24 November the concentration of the mottle strains in *D. tatula* was estimated serologically. Expressed sap was clarified by heating to 55° C. for 10 min., and the concentration of virus estimated by the virus end-point method. The *B* sap had an end-point dilution of 1 in 1280, while the other five strains gave an end-point of 1 in $\sqrt{2} \times 1280$.

ECOLOGICAL EVIDENCE FOR STRAIN RELATIONSHIP

Very little information has ever been obtained concerning the origins and distribution of strains of virus *X* in the field. An investigation carried out at Arran indicates the type of evidence which could be obtained.

The stocks of many of the standard varieties (e.g. Pepo, Great Scot, Arran Banner, Arran Peak) used by the late Mr Mackelvie for breeding stock and for comparison with seedlings were found to be 100% infected with symptomless strains of virus *X*. Most of the Arran seedlings were found to be similarly infected. As the stocks of all these seedlings passed one or more seasons in contact with the standard varieties, it seems likely that the strains of virus present in the newer varieties such as Arran Viking are fairly closely related to the strains present in the standard varieties used at Arran.

DISCUSSION

The work described above amply confirms the view that a very large number of strains of virus *X* exist. Salaman's six types, or, indeed, the strains described in the present work, are no more than an indication of the range that may occur.

Symptoms. There was no correlation between serological relationship and symptoms. The significance of symptom differences between strains is difficult to

evaluate. The division of strains into 'mottle' and 'ringspot' groups is largely arbitrary. Thus T.B.R. *X* is, in some respects, intermediate between the two groups, and environmental conditions can have a marked effect on symptoms produced. The fact that *Cyphomandra betacea* selects out 'ringspot' strains from a mixture indicates that the differences in the strains which produce the 'mottle' or 'ringspot' may be correlated with the demands made upon the plant for multiplication.

A difference in symptoms between two isolates of a virus, under standard conditions of host-plant genotype, method of inoculation, and environment, certainly means that two different strains are involved. In fact this is probably the most sensitive test available for detecting different strains, particularly if comparative tests are carried out over a range of environmental conditions. However, it is often irrationally assumed that a big difference in symptoms indicates a big difference in virus, and vice versa. The greatest amount of confusion has arisen in connexion with reactions on potato varieties. The following lines of evidence suggest that the ability to cause top necrosis in potatoes may be possessed independently of other characters, and by strains differing little in other ways:

(1) From comparison of U.T.D. Streak and *B* from Duke of York it is evident that the ability of the virus to cause top-necrosis in such varieties as President is possessed independently of serological relationship (Matthews, 1947).

(2) The ability to cause top-necrosis is possessed independently of symptom expression in other plants, for although there is a reasonably good correlation between symptoms in tobacco, etc., and in potato, it is not possible to predict from symptoms in tobacco whether a strain will cause top-necrosis in any particular potato variety.

Norval (1938), working with a series of tobacco-mosaic virus mutants, obtained more clear-cut evidence that, for this virus, different characters may be possessed independently by different strains. For instance, the ability to destroy tissues in tomato and tobacco appeared to be independent of the ability to become systemic.

(3) It was first shown by Bawden (1936) that in Up-to-Date potato the 'normal' and 'streak' strains existed together. On the evidence presented earlier it is at least probable that this mixture of strains arose by fairly recent mutation, and the component strains are therefore likely to be closely related. On this view, the gain or loss of the ability of the virus to cause top-necrosis in a given potato variety would be a fairly small change.

Serological tests. The technique used could have been made more sensitive by the use of stronger virus solutions for absorption and of antisera of higher titre. Had this been done it is almost certain that further smaller differences between the strains would have been detected. Also, if a larger number of serologically distinguishable strains had been used, a larger number of distinct antigenic fractions could have been detected. Thus there is no reason to suppose that each fraction labelled in the formulae represents a single antigenic structure.

The fact that the $X^{L}M$, $X^{H}M$, Dakota *M* and U.T.D. *M* isolates all had very

small amounts of severe type strains present could account, possibly, for the failure to show that the ringspot strains had any antigenic portions not possessed by the corresponding mottle strains. The presence of this contamination would, however, not affect the reverse result.

Apart from the single case of U.T.D. *R* antiserum absorbed with T.B.R. *X* virus, the results of the reciprocal cross-absorption experiments are internally consistent. It is considered that, as far as they go, the antigenic formulae derived give a sound indication of the antigenic relationship between the strains. The X^{II} group, T.B.R. *X*, and *B*, each have an antigenic fraction not possessed by any of the other strains tested. This allows the possibility of preparing an antiserum specific to one or a few strains (at least within the group tested). As a test of this a portion of X^{II} mottle antiserum was fully absorbed with X^{I} mottle virus. The antiserum thus prepared was used to identify correctly, by a single precipitation test, the two X^{II} strains studied, from a randomized group of samples of sap, two of which contained X^{II} strains, the other four being Dakota and X^{I} strains. The use of such strain- or strain source-specific antisera would greatly facilitate the study of strain populations in the field.

The results obtained with these ten strains show how different results could be obtained by different workers, if only about three or four strains were used, in this type of experiment. The extent of the difference obtained would depend largely on the strains which happened to be examined. Thus if only the group of X^{I}, U.T.D. and Dakota strains had been studied it might have been concluded either that strains of virus *X* were all closely related serologically, or that the technique was not sufficiently sensitive. Opposite conclusions could have been reached if only *B*, T.B.R. *X* and U.T.D. *M* had been examined.

Strains might differ not only in the kinds of antigenic structure they possess but also in the relative amounts of these. Table 3 indicates that $X^{II}R$ contains more of the X^{II} specific *h* fraction than does $X^{II}M$. A difference of this type could be detected by means of cross-absorption experiments between a number of strains. The serological relationships established appear to bear no relation to the symptoms produced by the strains, but there is an indication (with the X^{II} group) that strains from the same source regardless of symptoms may be more closely related to each other serologically than to strains from other sources.

Cross-protection in tobacco and Datura tatula. The six severe strains used reacted similarly with each of the mild strains. However, T.B.R. *X* and *B* differed from the other four mild strains in failing to afford complete protection against the severe strains used. This was not due to a difference in concentration of virus as far as could be detected.

The result of Smith (1946) with A.P. *X* and T.B.R. *X* is confirmed. The various results obtained by other workers may be due to their having worked with different strains, some of which afforded complete protection, and others which did not.

It is well established that cross-protection is a phenomenon occurring between

related strains, presumably connected with competition for available materials or sites for multiplication. T.B.R. *X* and *B* appear to differ fairly widely from the other ten strains used in some properties connected with the demands made upon the host cell, and such differences are considered more fundamental than, say, differences in type of mottle.

Among the strains of virus *X* examined, T.B.R. *X* and *B* differed from the other strains in three important respects:

(i) These two were the only mottle-type strains which did not give rise to any detectable mutants.

(ii) They both had strain-specific antigenic fractions and also shared a fraction not possessed by any other strains.

(iii) They did not give complete cross-protection in *Datura tatula* or tobacco against the ringspot strains tested. All other mild strains gave complete protection.

Thus there was a correlation between the degree of protection afforded in the host plant, and serological relationship.

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EXPLANATION OF PLATE 11

Cross-protection tests in *Datura tatula*. *a*. Partial breakdown in protection: Veinbanding mottle due to the *B* strain with necrotic local lesions of the second strain (Dak. *R*). *b*. Protection complete: *D. tatula* infected with X^BM, showing no symptoms, but completely protected against the severe strain (Dak. *R*). *c*. Control: Healthy *Datura* inoculated with Dak. *R*.

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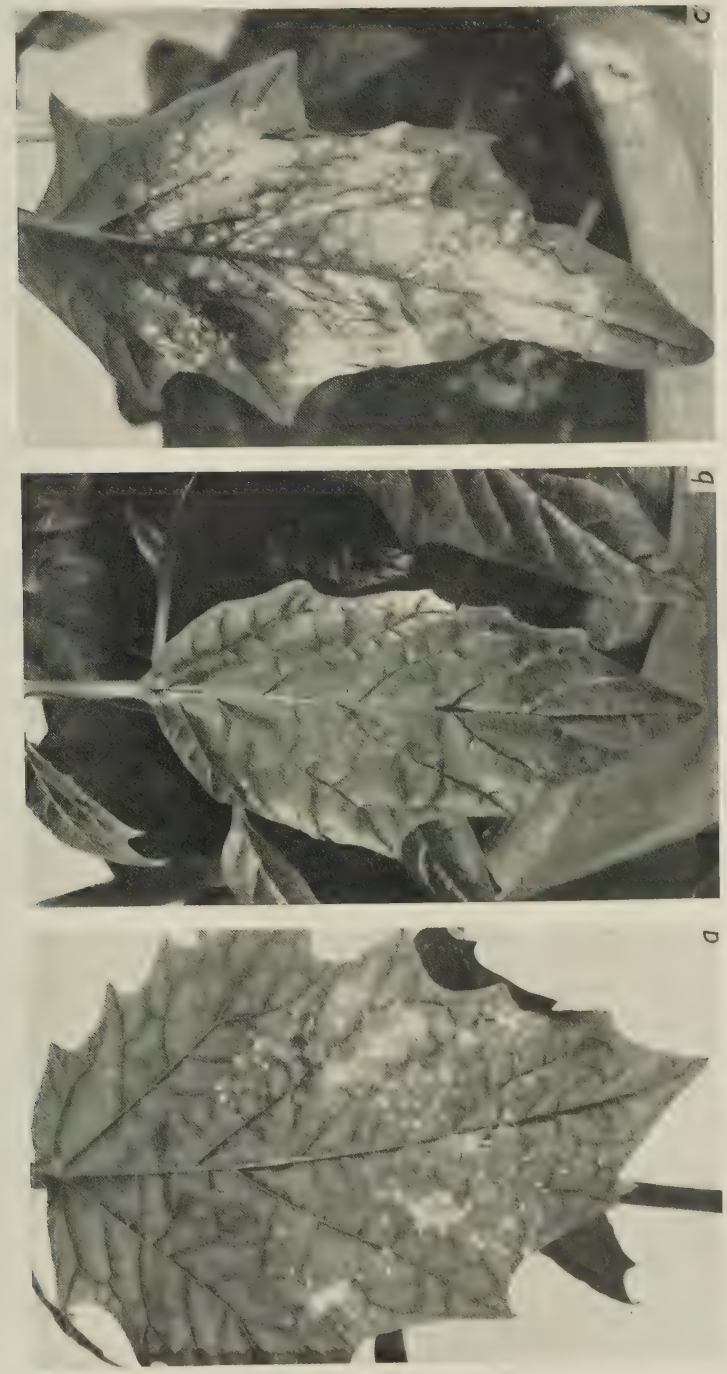

MATTHEWS—*Studies on potato virus X* (II)

THE SPATIAL DISTRIBUTION OF INSECT-BORNE PLANT-VIRUS DISEASES

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Various workers have proposed formulae to express the spatial distribution of insect-borne diseases. All the published data examined, as well as the Rothamsted data for the spread of rugose mosaic and leaf-roll from point sources in potato crops, were fitted as well by the simple empirical expression $\log I = a + bx$ as by more complex expressions (I = number of infective punctures at a distance x from the source after a given time, and a and b are constants for any one given set of field conditions). It is suggested that distances should always be given in metres, in order to give comparable results from one investigation to another. In the analysis of data on rugose mosaic and leaf-roll in different years, it is shown that a and b vary independently.

Introduction

Many procedures used for controlling plant diseases depend on the spatial separation of diseased and healthy plants. Control by isolation or roguing calls for knowledge of the manner in which the incidence of a disease, expressed as number of lesions or infected plants per unit area, decreases with increasing distance from the source; and in considering the gradient of a disease, the three main dispersal routes need separate consideration. These routes are: (1) dispersal of dry air-borne spores; (2) rain-splash dispersal of slime-spored fungi and bacteria; and (3) dispersal by insect vectors. The present paper attempts to evaluate the form of the spatial distribution of two insect-borne plant-virus diseases, in order to obtain suitable measures of the effects of treatments employed in studying them. It is based on an analysis of data on the spread of rugose mosaic and leaf-roll in potato crops at Rothamsted over the years 1943–6.

Review of earlier work

Frampton, Linn & Hansing (1942), working with potato yellow dwarf, considered the spread as a function of both time and distance. Expressing the density of insect attack in the form of differential equations, and solving under the restriction of certain limiting assumptions, they arrived at the expression

$$I = Kt \exp(st - \sqrt{s}\, x),$$

where I = number of plants that have become infected in time t, x = co-ordinate in the direction of insect flow, K = average distance an insect will move in the x direction in unit time, and s = constant of integration. The expression deals with spread at right angles to an infected strip, and not from a point source. The

assumptions involved in the derivation of this equation were that the movement of the insects involved was random, that the number of plants that became infected was proportional to the number of plants that had been fed upon, and that spread from plant to plant within the field was of small importance. The boundary conditions of integration were that the insect population in the newly ploughed field was negligible, that the insect reservoir was not substantially depleted during the course of the experiment, and that the effects at the ends of the fields could be neglected.

Frampton *et al.* (1942) observed that the integrated form of the equation demands linear relations between $\log I$ and x (t constant), and between $\log I$ and $(t+\log t)$ (x constant), though the latter is only true for $s=1$. The observed data were in good agreement with these demands.

Zentmyer, Wallace & Horsfall (1944), studying the radial spread of Dutch elm disease from a point source (*Ceratostomella ulmi* (Schwarz) Buisman) by the European elm-bark beetle (*Scolytus multistriatus* Marsh.), supposed that the problem could be treated by applying the 'dosage-response' principle (following earlier examples by Dimond, Horsfall, Heuberger & Stoddard (1941) and Heald (1921)), 'response' being represented by the numbers of newly infected elms, and the distance of the new infections from the point source constituting the 'dosage' factor. Regressions were fitted by the usual probit method, and a rectilinear relation was found between the logarithm of the distance and the probit of the percentage infection. We find, however, that the same data could equally well be fitted (in the sense that there is no statistically significant departure from such a relation) by a linear relation between distance and log percentage infection. And since *distance* (rather than, say, log distance or $\sqrt{}$distance) is merely an arbitrary dosage metameter, the probit approach seems unnecessarily elaborate, although it fits.

Wadley & Wolfenbarger (1944) also studied the radial spread of the European elm-bark beetle from a point source, in its role as a vector of the Dutch elm disease. Taking the percentage of twig crotches attacked as a measure of insect density in two infestations, they found that the data were well fitted by regressions of the form

$$y=a+b\log x \quad \text{or} \quad y=a+b\log x+c/x,$$

where $y=$percentage crotches attacked, $x=$distance from the source of infection, and a, b and c are regression constants. Log $y=a+bx$ fitted less closely, but was not shown to be significantly inferior.

Wilson & Baker (1946) concluded that for the spread of endive yellows by leafhoppers (Linn, 1940), yellow dwarf of potatoes by leafhoppers (Frampton *et al.* 1942), and Dutch elm disease by bark beetles (Zentmyer *et al.* 1944), the relationship between the incidence of infection and distance from the source was, in some respects, similar to that which they found for the spread of air-borne spores, viz. incidence proportional to $1/x^2$ ($x=$distance from source), or to $1/(x+a)^2$, where a is a constant depending upon the conditions of dispersion. However, as already

pointed out above, the second and third sets of data could equally well have been fitted by an expression of the form $\log y = a + bx$.

Wolfenbarger (1946) considered that the dispersion of a wide variety of organisms, including bacteria, spores, seeds, pollen and insects, was fitted by a regression of the form

$$E = a + b \log x \quad \text{or} \quad E = a + b \log x + c/x,$$

where E = expected number of organisms at distance x from some fixed origin. Often, however, it is apparent that any one of a number of other types of regression such as $\log E$ on x, or E on x, for example, could have been fitted equally well; that is, there would have been no statistically significant departure from any one of certain other relationships, especially as the observed data (pairs of observations of number of organisms, and x) often comprised only 3–5 points.

Bateman (1947), working on insect pollination, concluded that the frequency (n) of flights of distance x from a point source should best be expressed by an equation of the form $\log n = a + bx^p$; and from a study of the flights of hive bees, solitary bees, and hover flies he found that $p = \frac{1}{2}$ gave a better fit than $p = 1$ which in turn gave a better fit than $p = 2$.

On the other hand, the proportion (F) of contamination by insect cross-pollination appeared to be fitted equally well by the expression

$$\log F = a + bx^{\frac{1}{2}} \quad \text{or by} \quad \log F + \log x = a + bx.$$

The factors involved in the spread of virus diseases are probably not identical with those involved in pollination, and it would not be reasonable to assume that a mathematical relationship holding for one would necessarily hold for the other.

Much of the past literature may fairly be summarized in stating that, although a number of theories and mathematical relationships have been proposed, for any given insect or disease the evidence in favour of any particular form of relationship, in contrast to any other of a number of possible and plausible forms, is either not well established or totally inadequate.

An empirical analysis

The present knowledge of field conditions is insufficient to give adequate fundamental guidance, and it is not possible to do better than make a rough guess at the mathematical form of the distribution of the insects or of the degree of plant infection. Among other additional complications, there is the 'movement' parameter, as described by Neyman (1939); but we have no adequate field data for its evaluation. Thus the possible approaches rest on too many assumptions which, though perhaps reasonable enough, are unfortunately unproven (or unprovable), and factors which cannot be evaluated. The only possible solution remaining is one that is largely empirical. The general trend is a rapid decrease in infection with increasing distance

from the source; and the relationship is definitely curvilinear. Following Bateman (1947), it was considered that the best form of equation was

$$\log_{10} I = a + bx^p,$$

where I = number of infective punctures at distance x from the source after any given time, and a, b and p are constants for any one given set of field conditions. (The numbers of infective punctures are estimated by means of the Thompson Multiple Infection Transformation, $I = \log_e \left(\frac{N}{N-D}\right)$, where D = number of diseased plants out of a total of N (Thompson, 1924). Very few of the previously published data are in significant contradiction with this general form. Further, from examination of the field data described below, it appeared that $p = 1$ gave a generally better fit than did $p = \frac{1}{2}$ or 2. However, these data were too few and too variable to show any statistically significant departure from the expressions with $p = \frac{1}{2}$ or 2. Use of the Multiple Infection Transformation (Thompson, 1924; Gregory, 1948*b*) also made little appreciable difference; with such variable data its effect must necessarily remain relatively unimportant except where very high percentages are concerned. Its use was preferred, however, on general grounds, and it removed the occasionally observable anomaly of extrapolation back to $x = 0$ giving a percentage infection greater than 100.

Analysis of Rothamsted field data

Having decided on the form of equation to be used, the Rothamsted data were analysed by normal regression methods, to give estimates of the average number of infective punctures at 1 m. (taken as an arbitrary standard distance), and of the infection gradient.

The data are from the experiments of 1943–6 on the effect of roguing virus-diseased potatoes, described in detail in earlier publications (Doncaster & Gregory, 1948, chap. 7; Gregory, 1948*a*). Potato tubers with rugose mosaic or leaf-roll were planted in known positions in large plots of healthy Majestic potatoes, and at the end of the season adjacent plants were sampled by the 'infector unit method'. Single tubers were taken from each of the five originally healthy plants on either side of the infector and in the same row, and were planted in the following year to ascertain whether they had become infected. From the data obtained the probability of a plant (as distinct from a 'hill') in the first five positions on either side of the infector becoming infected could be estimated. Since the samples were not very large, the distinction between rogued and unrogued plots has been ignored, and analysis based on the pooled results. The data are summarized in Table 1, where plant positions on one side of the infector are numbered 1 and those on the other side 2. It will be seen that differences in amount of infection on the two sides have usually been small.

The calculated values of I are also given in Table 1. Since the number of plants

studied varied from year to year, I has been expressed as the number of infective punctures per 100 plants, and is therefore given by $100 \log_e \left(\frac{N}{N-D}\right)$, where D is the number of diseased plants out of a total of N. x, the distance from the source, is in units of 18 in. in 1943–5, but 2 links (= 15·8 in.) in 1946.

TABLE 1. *Virus infections on either side of point sources in potato crops at Rothamsted*, 1943–6

		Rugose mosaic				Leaf-roll			
		Infected plants (D)		No. infective punctures/100 plants (I)		Infected plants (D)		No. infective punctures/100 plants (I)	
Year	Distance (x)	1–5* 1	6–10* 2	1–5 1	6–10 2	1–5 1	6–10 2	1–5 1	6–10 2
1943 (N=80)	1	39	35	67·0	57·6	37	44	62·2	79·9
	2	15	11	20·7	15·0	23	26	34·1	39·4
	3	7	2	9·2	2·5	18	13	25·6	17·7
	4	2	4	2·5	5·1	14	7	19·3	9·2
	5	3	2	3·9	2·5	9	9	12·0	12·0
1944 (N=96)	1	54	50	82·7	73·7	76	82	156·8	192·5
	2	22	23	26·0	27·4	62	51	103·8	75·8
	3	11	25	12·2	30·2	41	50	55·7	73·7
	4	11	7	12·2	7·6	29	34	35·9	43·7
	5	5	4	5·3	4·1	16	19	18·2	22·1
1945 (N=64)	1	24	25	47·0	49·5	30	28	63·3	57·6
	2	5	4	8·3	6·4	12	16	20·7	28·8
	3	4	2	6·4	3·2	6	12	9·9	20·7
	4	4	2	6·4	3·2	6	8	9·9	13·4
	5	0	1	0†	1·6	6	4	9·9	6·4
1946 (N=76)	1	15	17	21·9	25·3	38	27	69·3	44·0
	2	7	9	9·7	12·7	23	11	35·9	15·7
	3	3	1	3·9	1·4	13	16	18·7	23·7
	4	5	4	6·7	5·3	8	7	11·1	9·7
	5	4	7	5·3	9·7	4	9	5·5	12·7

* These numbers refer to the position, 1 being a replicate of 2, on the opposite side.
† For analytical purposes this figure has been replaced by 0·5, since $\log 0 = -\infty$.

From the data given in Table 1, the constants a and b in the equation

$$\log_{10} \hat{I} = a + b\,(x - \bar{x}),$$

(where $\hat{I}$ = the value of I expected on the basis of the regression), were determined by ordinary least-squares procedure. (With more extensive data the use of the maximum likelihood solution would have been worth while, but would have led to conclusions similar to those given by the simpler method adopted here.) The results, with the corresponding standard errors, are given in Table 2, b being expressed in units of (metres)$^{-1}$. It will be noted that multiplication of b by $\log_e 10$ gives the *relative* decremental rate, infections/metre/infection—'infections' signifying effective

punctures per 100 plants. In addition, the table gives values for $\hat{I}_1$, the expected number of infective punctures per 100 plants at 1 m. from the source.

TABLE 2. *Infection gradients for rugose mosaic and leaf-roll spreading from point sources in potato crops at Rothamsted*, 1943–6

(All standard errors based on 3 D.F.)

	Rugose mosaic								Leaf-roll							
	1943		1944		1945		1946		1943		1944		1945		1946	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
a	1·02	0·89	1·25	1·26	0·78	0·74	0·89	0·87	1·41	1·36	1·75	1·80	1·22	1·29	1·29	1·26
S.E.	0·10	0·15	0·07	0·07	0·15	0·13	0·10	0·22	0·02	0·07	0·01	0·10	0·09	0·02	0·01	0·08
b (m^{-1})*	0·74	0·70	0·59	0·67	0·89	0·72	0·34	0·31	0·37	0·50	0·51	0·46	0·42	0·49	0·67	0·32
S.E.	0·16	0·23	0·10	0·11	0·23	0·19	0·17	0·40	0·03	0·11	0·02	0·07	0·14	0·04	0·01	0·14
$\text{Log}_{10}\hat{I}_1$	1·29	1·15	1·47	1·51	1·11	1·01	0·96	0·93	1·55	1·54	1·94	1·98	1·38	1·48	1·43	1·33
S.E.	0·12	0·17	0·08	0·08	0·17	0·14	0·11	0·24	0·02	0·08	0·02	0·15	0·11	0·03	0·01	0·08
$\hat{I}_1$ (nearest integer)	19	14	30	32	13	10	9	9	35	35	87	95	24	30	27	21

* b is negative in all cases; sign omitted for brevity.

DISCUSSION

It will be observed that the variation of $\hat{I}_1$ between years is considerably greater than that between the replicate sets of positions within years. Thus, in analysis of variance form (in units of $\log_{10}\hat{I}_1$):

	D.F.	Sums of squares	Mean squares
Diseases	1	0·6400	0·6400**
Years	3	0·7382	0·2461**
Diseases × years	3	0·0127	0·0042
Residual	8	0·0269	0·0034
Total	15	1·4178	

The variation between years is seen to be highly significant, as also is the difference between the two diseases. It follows that, though the nature of the factors causing this variation may not be properly understood, the present analytical procedure can at least detect such variation, and provides a method for examining the effects of such factors as may be discovered in the course of more extensive field work. It will also be observed that the interaction diseases × years is negligible; i.e. the *form* of the variation between years is similar for the two diseases, suggesting the existence of factors which affect the spread of the two diseases in a similar manner.

The corresponding figures for gradient (b) are somewhat irregular, and show no such marked effects as are evident with $\hat{I}_1$. The average gradient appears to be more or less constant, except in 1946, where the replicate values for rugose mosaic are both low, and those for leaf-roll inconsistent. Examination of the figures in Table 1 suggests that this may be due to a generally low level of infection in 1946, with infection from outside (and other similar sources of error) tending to mask the gradient that would otherwise have been observed.

In the light of these results, it is interesting to consider the meaning of the original empirical expression, $\log I = a + bx$. If b is constant, it follows that the aphids are behaving in the same way from year to year, and a simple proportionate relationship

is retained for the intensity of infection at any given distance, a constant difference in log I being equivalent to a constant ratio for I.

The spread of a virus disease over a crop can thus be analysed into two parts, measured by the height and slope respectively of the graph of log intensity on distance; and these two components of virus spread vary independently. In any further study of gradients shown by plant virus diseases attention will have to be paid to the following points:

(1) Experiments will have to be designed to yield many more pairs of observations of numbers of infections (or infective punctures) and distance from source. With potatoes the number of observations is limited by the small distance from the source at which these numbers can be measured with reasonable accuracy, and by the requirement that potato plants must be at least one foot apart.

(2) The geometrical form of the source will affect the gradient (Gregory, 1948*b*). With a point source the slope of the regression line is expected to be steeper than with a line or an area source under similar conditions. When necessary, allowance will have to be made for this factor before comparisons between gradients are legitimate.

(3) When more than 20% of the plants are infected it is essential to make the multiple-infection transformation.

Fitting curves to dispersal gradients may prove to be applicable to many kinds of organism, but any common principles will remain obscure unless common units of distance are used. Parameters for gradients already published contain hidden units of distance varying from inches to nautical miles. In order to be able to compare gradients we suggest tentatively that distances should be given in metres, that intensity should be calculated at 1 m. from source ($\hat{I}_1$), and that slope, b, should be calculated per metre. It is useless to give only the relative distances at which observations were made, as has sometimes been done, because this ignores the fact that a particular slope characterizes a particular distance from the source. The present study is an attempt at a more systematic approach to the measurement and comparison of virus disease gradients. Although a better fit to individual sets of data could be obtained with more complex expressions, it will be advantageous to apply this approximate general formula. The parameters a and b obtained in different diseases, localities, seasons, etc., can then be compared, for the elucidation of general principles which would remain obscure if each set of data received individual treatment.

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OBSERVATIONS ON APPLE CANKER

III. THE ANATOMY OF THE STEM CANKER

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(With Plate 12 and 5 Text-figures)

The development of cankers caused by *Nectria galligena* on apple is described; the pathogen exploits all the tissues outside the xylem and will also penetrate the xylem to an appreciable depth, invading the xylem parenchyma, vessels and fibres.

Spread in the peripheral tissues is checked to some extent by successive barriers laid down by wound phellogens, but the barriers are eventually passed by the mycelium. There appear to be two distinct ways in which these barriers are passed: there may be direct penetration which appears to be associated with an aggregation of the mycelium into blocks, and symptoms suggestive of mechanical rupture; or there is an alternative route in which the pathogen grows in the lumen of the fibres and emerges behind the barrier.

In the xylem the spread of the pathogen is checked by tyloses and gumming in the vessels, and by gumming in the parenchymatous tissues, but in the fibres there appears to be no defence mechanism, and the spread beyond the gum barriers was usually recorded in the xylem fibres. It is suggested that the presence of the pathogen in the xylem fibres might provide the explanation of the formation of cankers on partially healed pruning cuts, and this type of canker is described in some detail.

The development of the canker depends on the balance between the development of the pathogen and the resistance of the host, and there is some discussion on the relation of this balance to the control of the disease.

Introduction

A general description of the canker disease of apples has been given in the first paper in this series, which also included a description of the causative fungus *Nectria galligena* Bres. (Munson, 1939). The earlier papers were concerned primarily with the early stages of infection and the prevention of the disease: Munson (1939) investigated the conditions governing the discharge and the germination of the ascospores and conidia and Marsh (1939) the conditions governing the infection of pruning cuts and leaf scars, and the methods by which the infection could be combated. The present contribution aims at describing the behaviour of the pathogen in active cankers, and discussing the relation of the development of the cankers to their control.

The serious study of *Nectria* cankers probably dates from about 1880, in which year Goethe published a study of canker disease on apples and Hartig a study of a similar disease on a selection of broad-leaved trees, particularly copper beach. Both authors studied the anatomy of the lesions and the tissues invaded.

Hartig also suggested that the concentric rings typical of old cankers were the result of alternation in the growth period of the fungus and the host. More recent contributions to the study of the anatomy of the lesions have been published by Voges (1914), who described the tissues occurring in cankers on fairly old apple stems, and by Wiltshire, who described the early stages in the formation on apple of cankers resulting from the infection at leaf scars (1921) and from the infection of lesions formed by the scab fungus *Venturia inaequalis* (Cooke) Wint. (1922). Detailed descriptions of the disease and the anatomy of the lesions have also been published by Zeller (1926) who worked with pomaceous fruit, and by Ashcroft (1934) who was primarily interested in black walnut (*Juglans nigra* L.).

Nectria galligena is able to enter the host only through wounds, and Wiltshire (1913) found that the pathogen was successfully confined in the cortex if the wound was shallow, and that in order to give rise to a canker the wound had to penetrate nearly to the wood. In the field, infections are probably most commonly established in leaf scars (Wiltshire, 1921), pruning wounds, splits and frost injury. Wiltshire (1919) found that infection of woolly aphid galls was relatively low, only about 2%, and that it was only burst galls which became infected, since the fungus appeared unable to penetrate through the insect punctures. Infection may also follow injuries caused by other pathogenic fungi such as *Venturia inaequalis* (Wiltshire, 1922) and *Neofabrea malicorticis* (Zeller, 1926).

After infection has been established, the spread of the lesion can be seen by the discoloration of the bark which later becomes blackened and sunken. The cortical and phloem tissues below the bark dry out, separate from the wood, and later crack and break away, exposing the xylem. The fructifications of the fungus are seen on these dead tissues. Even in these early stages a zonation in the spread of the lesion has been noticed (Zeller, 1926). As the pathogen spreads, the host tissues are stimulated to extra activity, with the result that there is a marked swelling of the shoot in the canker region. There is usually a strong callus lip at the edge of the canker. Cankers spread more rapidly along the length of the stem than around its circumference; they are, therefore, generally oval in shape; in time they may girdle the shoot and kill it. In old cankers, which have been developing for more than one season, there is usually a marked concentric ridging in the exposed wood; this appears to be caused by seasonal differences in the relative growth rates of the pathogen and the host; in the apple, active wound regeneration appears to be confined to a few months during the growing season (Crowdy, 1949), while Munson (1939) has shown that development of *Nectria galligena* is continued throughout the year. During the growing season there is a vigorous formation of host tissue which also provides a distinct check to the development of the pathogen with the result that a ridge is formed, while during the dormant season there is probably little tissue laid down, and rather less check to the spread of the pathogen; one of these ridges is shown in Text-fig. 3.

N. galligena appears able to exploit all the tissues to which it penetrates, but it

also appears that the mycelium is unable to penetrate living tissues directly, and these are killed in advance of the mycelium by secretions of the fungus (Wiltshire, 1922). In the cortex and phloem the hyphae in the first instance are mainly intercellular though in later stages the cells are occupied; the mycelium grows especially strongly in the phloem region. The mycelium appears to enter the xylem by way of the medullary rays (Goethe, 1880), but once within the xylem the hyphae grow freely within the vessels and tracheids. The pathogen appears to be unable to penetrate the lignified walls of these tissues, and there is no tendency for the wood to disintegrate; spread from one cell to the next is by way of the pits.

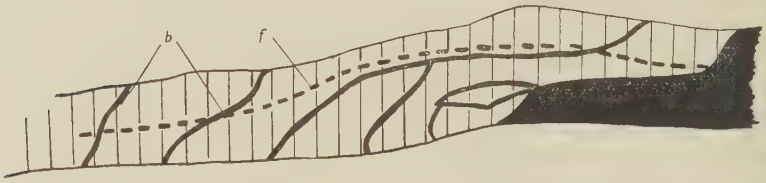
The reaction of the host to invasion is marked; the spread in the xylem vessel is restricted by tyloses and gum barriers, and phellogens bound the lesion in the peripheral tissues, appearing temporarily to prevent spread. Under the stimulus of the infection a characteristic wound callus is laid down which consists of two zones: wound wood which is a hard tissue built up of tightly packed isodiametric cells in the areas adjacent to the cankers, and which shades gradually into normal xylem tissue as the distance from the active canker increases; this tissue, which appears to be a modification of normal callus tissue characteristic of canker, gums readily and the gum barriers offer marked resistance to the spread of the pathogen. Outside the woody zone there is a zone of soft parenchymatous tissue which is only distinguishable from the normal peripheral tissues by the absence of fibre bundles, and which reacts to infection in the same way as the normal phloem and cortex.

It will be seen from the foregoing description that the canker lesion is the result of a balance of two opposing effects, the spread of the pathogen and the reaction of the host, and that when the host is active it may show marked powers of resistance. Some of the methods by which the pathogen overcomes the resistance of the host are described below.

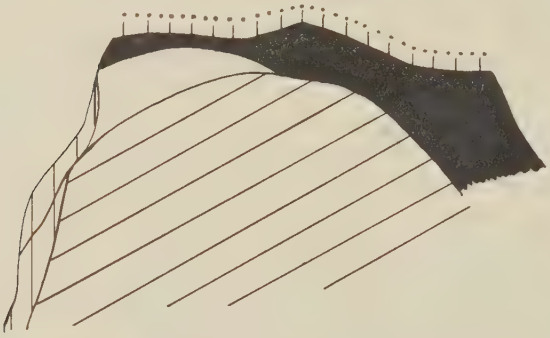
Experimental methods

The observations on which the following description is based were confined to naturally occurring cankers on apple. In the main, two very susceptible varieties, Worcester Pearmain and Cox's Orange Pippin, were examined. The cankers were collected at various times of the year and were examined in longitudinal and transverse section cut as far as possible through the point of infection, which usually seemed to be the dead base of a lateral shoot near the centre of the canker. The sections were cut either from the fresh material or after the tissues had received a preliminary softening treatment in which they were waterlogged by boiling for about 15 min., immersed in a mixture of equal parts of glycerine and methylated spirits for 3 days, and stored in a 10% glycerine-methylated spirit mixture (Department of Scientific and Industrial Research, 1946). This softening treatment had no effect on the anatomy of the lesions or the ease with which the pathogen could be differentiated in the tissues, and the softened tissues were easier to handle and section than the fresh.

Text-fig. 1. Longitudinal section of canker on Cox's Orange Pippin.



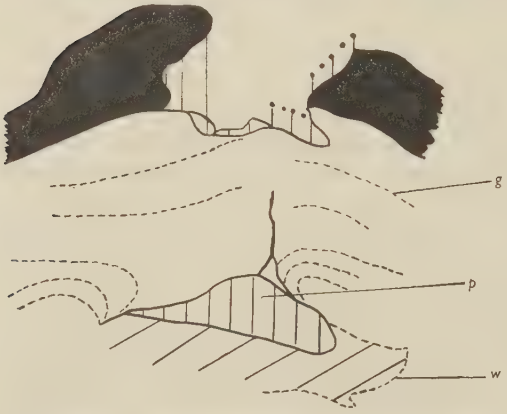
Text-fig. 2. Longitudinal section of canker on Worcester Pearmain.



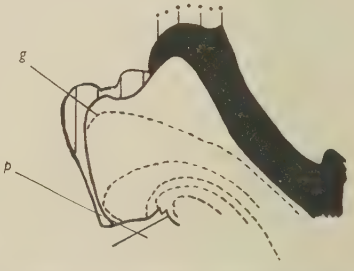
Figs. 1, 4 and 5
100 mm.

Figs. 2 and 3
50 mm.

Text-fig. 3. Transverse section of canker illustrated in Text-fig. 1.



Text-fig. 4. Longitudinal section of latent canker on Worcester Pearmain.



Text-fig. 5. Transverse section of half the canker illustrated in Text-fig. 4.

b, wound barriers; *f*, fibre bundle; *g*, growth rings; *p*, base of pruned stem; *w*, boundary of gummed area.

The most satisfactory stain combination was found to be safranin and picro-aniline-blue, though the schedule suggested by Cartwright (1929) was modified to some extent. The sections were allowed to soak in the picro-aniline-blue for about 15 min. to allow good penetration into the tissues, and were then warmed for about 1 min. to allow a rather heavy staining. This stain gave excellent differentiation of the mycelium in the lignified tissues, and in the peripheral tissues differentiated clearly between the healthy and the necrotic tissues.

The longitudinal sections of large cankers were usually cut in two or three parts and the final diagrams combined to give a complete picture of the canker.

The anatomy of the canker

Text-figs. 1 and 3 show diagrammatic transverse and longitudinal sections of a canker on Cox's Orange Pippin which was collected during December; the shoot was in its fifth year and the canker appeared to have originated early in the preceding season at the base of a lateral shoot. The diagrams show the general distribution of the host tissues which have been described above, and also the regions in which the mycelium was found. At other seasons of the year the general distribution of the tissues was the same, though at the height of the growing season the peripheral tissues tended to be more pronounced in the transverse sections. This transverse section (Text-fig. 3) shows clearly the first of the annular ridges, formed during the first growing season.

Spread in the xylem

The fungus develops readily in the xylem of the host in the vessels, fibres and medullary rays. Near the exposed surface of the wood in the lesion the hyphae are frequent and appear strong and healthy, but they become less frequent and more attenuated the farther they extend from the centre of infection; the spread in the vessels seems to be blocked effectively by tyloses and gumming fairly close to the original site of infection, and spread beyond the limits of the exposed xylem is usually in the lumen of the fibres. The pathogen may spread in the xylem beyond the limits of the lesion in the wound wood immediately overlying it. When this occurs there may be a slight gumming of the wound wood adjacent to the infected xylem, but there is no suggestion that the wound wood is infected from below. The mycelium has been traced in the fibres for distances up to 14 mm. behind healthy canker wood, but at these distances the hyphae are very poorly developed. The area of gummed wood which can be seen with the naked eye usually extends beyond the limits of the mycelium.

The wound wood adjacent to the canker is a closely packed tissue which appears to gum easily, and it seems that the pathogen can penetrate this tissue only with difficulty, although there is a slow spread. As can be seen from the diagrams the spread in this tissue lags far behind the spread in the peripheral tissues.

Spread in the peripheral tissues

The spread in the soft tissues outside the xylem seems to occur in a series of distinct stages. The presence of the pathogen stimulates the peripheral tissues to form a phellogen which gives rise to a barrier which contains the lesion for a period, but after a time the protection of the phellogen breaks down, and there is a further extension of the lesion which is in turn contained by a new phellogen. This process is illustrated in Text-fig. 2, which is a longitudinal section through part of a canker and shows a series of barriers which have been passed. The individual barriers are separated by relatively large blocks of tissue; the lesion spreads more rapidly on the outside, and this spread is associated with a fibre bundle (*f*). This rather erratic progress is probably the explanation of the zoning noted by Zeller (1926) in the early stages of canker formation. The effectiveness of the barrier in checking the spread of the mycelium will depend on the resistance offered to direct penetration, and also on the ease with which the pathogen can avoid the active host cells in its progress. The direct penetration of the barrier is not a simple process, since it has already been noted that the pathogen is unable to penetrate uninjured cells, and the clear demarcation between necrotic and healthy tissues indicates that the barrier possesses considerable powers of resisting the diffusion of the fungal toxins. It would appear that in the time which elapses between the formation of the barrier and its rupture, the newly invaded tissue is permeated by the pathogen which later builds large aggregates of mycelium near the barrier (Pl. 12, fig. 1, *A*), and in many of the cases examined the spread of the lesion appeared to be related to the presence of one of these blocks. The barrier near these mycelial aggregates frequently tears under the razor when a section is being cut (Pl. 12, fig. 1, *B*), which suggests that the growth of the aggregate imposes a mechanical strain on the host tissues, and that the penetration of the barrier is due to a mechanical rupture. On the other hand, it is possible that the damage is caused by an abnormal concentration of toxins produced by the aggregated mycelium. When the resistance of the phellogen has been broken, a relatively large block of tissue seems to be killed more or less at the same time, and this is again occluded by the formation of a new phellogen some way behind the other. It seems possible that there is an accumulation of toxins which is released suddenly when the barrier is broken and the toxins spread through the tissues until they are too dilute to cause further damage, the new phellogen being laid down where the damage ceases.

In addition to being able to penetrate the active phellogen in this way, *N. galligena* can avoid the active tissue by growing in the lumen of the phloem fibre cells. This provides a convenient and unimpeded path through the active layers to the soft tissues which lie behind. Pl. 12, figs. 2 and 3, illustrate typical signs of this spread through the fibres. The response of the host to the presence of the pathogen in the fibre is to form another wound phellogen which separates the diseased from the healthy tissue. These phellogens occur most commonly near the active edge of the

lesion, but they have been noted at distances up to 10 mm. inside the healthy tissue, and a search would probably reveal an even greater depth of penetration. When these deep penetrations occur the phellogens are found at isolated points along the fibre bundle which suggests that they are formed only where the mycelium emerges into the soft tissues. The actual depth of penetration is probably controlled by the relative speed with which the lesion can spread as compared with the rate of growth of the mycelium within the fibres; fibre spread is most obvious where the active spread of the lesion has been checked either by the application of growth substances or by the removal of the obviously diseased tissue.

Zeller & Owens (1921) and Zeller (1926) have described an unusual canker in pear which occurs in the United States of America, which from its early behaviour they call superficial canker. The early stages in the development of these cankers are characterized by a rapid spread of the disease in the outer layers of the peripheral tissues which is associated with a browning of the parenchyma surrounding the 'bast bundles', and Zeller was able to isolate *N. galligena* from these brown bundles. This browning of the bast bundles is strongly suggestive of the fibre spread recorded above.

The spread of the fungus seems to be essentially the same in the phloem and in the xylem fibres. In the early stages the mycelium always grows within the cells, and travels from cell to cell by way of the pits. During this stage the development of the hyphae seems poor and weak, especially in the phloem fibres. Pl. 12, fig. 4, illustrates mycelium in the lumen of the xylem fibre cells; in the later stages there is also a development of intercellular mycelium, the distribution of which is highly characteristic, since the hyphae grow spirally around small groups of fibres within the bundles. At times there may be two or three spirals of mycelium enclosing the same group of fibres giving a peculiar laced appearance to the tissue (Pl. 12, fig. 5). In the peripheral tissues this intercellular growth has only been observed in the necrotic parts of the lesion, but the same structure also occurs associated with the xylem fibres when the tissue shows no signs of disintegration, but again it would appear that the infections are of long standing. The illustration of intercellular development (Pl. 12, fig. 5) is taken from a section of xylem fibres, and it can be seen that there are no signs of the tissue disintegrating in spite of the heavy infection.

It is fairly clear from the structure of the tissues involved that the supplies of oxygen within the fibre cells must be extremely limited, and it was felt that some observation on the growth of *N. galligena* in anaerobic conditions would be of interest. In a preliminary growth trial spores of the fungus were sown in test-tubes half filled with 2% malt broth which had been sterilized in an autoclave, half these tubes being sealed with a layer of sterile paraffin wax after sowing. This test allowed a comparison between the behaviour of cultures in which a limited supply of oxygen was available at the exposed liquid surface, and others in which even this supply was excluded with wax. In the series without wax the fungus made fair growth, and there appeared to be little difference between the development of the submerged

colonies, whether they were formed near the top or the bottom of the tube. Even when the air had been excluded with the wax the colonies were formed freely, but in this case they were very small and poorly developed. It would appear, therefore, that the limited supplies of oxygen should not prevent the development of the intracellular hyphae in the fibres, though it might well prevent a strong mycelial growth, especially in the phloem fibre cells in which the lumen is small.

Cankers on healing pruning cuts

The development of the pathogen in the fibres may also explain the occurrence in the field of cankers which are associated with partially healed pruning cuts. This type of canker was described by Moore (1934), who suggested that moisture collected in the hollow left by the closing callus ring provided conditions which were favourable for the development of the pathogen. This explanation is not entirely satisfactory, since a partially healed pruning cut will have been exposed for at least one growing season, and Swarbrick (1925) has shown that in wounds of this type the xylem was completely plugged during the first growing season after wounding; further, before complete plugging can be expected to have taken place wounds in apple develop a marked resistance to infection with *N. galligena* (Marsh, 1939), and a similar resistance has been shown in wounded plum against invasion by *Stereum purpureum* Pers. (Brooks & Moore, 1925). These observations suggest that unless a wound becomes infected soon after it is made it is unlikely to develop into a canker, and if this is the case cankers of this type probably arise from infections which have remained dormant in the wound, and can fairly be described as latent cankers.

The characteristic feature of these latent cankers is that some normal healing has taken place over the wound before the development of the obvious canker symptoms; this distinguishes them from the cankers described above in which the symptoms develop soon after wounding. The difference is usually quite obvious in the field, particularly when healing is fairly far advanced, and it also shows very clearly when the canker is sectioned, since the wound wood formed while normal healing is in progress can be distinguished easily from that formed under the stimulus of *Nectria galligena* infection. Further, when cankers develop in the first season at the base of an infected lateral they spread in all directions, leaving the dead lateral near the centre of the canker, while in the latent type spread is frequently from one side of the healing wound. Latent cankers occur fairly frequently, especially on susceptible varieties, and they seem to be most common on the larger branches of the trees—a distribution which tends to increase their importance since individual cankers may affect large sections of the tree. In many cases the canker symptoms become obvious fairly early in the healing of the wound when the cut surface of the lateral is still obvious, but cases have also been noticed in which the wound has practically healed over, and the presence of the pruned lateral could only be detected when the branch was split. In all cases splitting revealed severe discoloration in the base of the pruned lateral.

Normally, pruning cuts heal by the formation of pads of callus tissue at their edges which gradually grow inwards and cover the surface of the wound. These callus pads have two obvious tissue zones: a hard woody core surrounded by an outer soft parenchymatous zone. The main development of the soft zone is on the outside of the wound, but a little is also formed on the side adjacent to the cut surface, and this is crushed as the pad develops. When the pads meet over the wound they fuse, and this fusion also appears to be accompanied by some crushing. The healing of latent cankers appears to follow this normal course until the infection begins to develop.

Text-fig. 4 shows a longitudinal section through a typical latent canker on Worcester Pearmain collected in May in which healing is practically complete, and Text-fig. 5 a transverse section through one side of the same canker. Text-fig. 4 is slightly excentric and healing was not quite as complete as would appear from the diagram; this was difficult to avoid if the same canker were to be sectioned in both directions. The callus pad showed five growth rings, but it is difficult to judge age precisely in the confused callus tissue, and it seems probable that the first three rings were all part of the first year's growth and the actual age of the wound was 3 years. The surface canker appeared to have started development in the third year. The distribution of the infected tissues within this canker was characteristic; part of the base of the pruned branch was infected, and mycelium could also be distinguished in the parenchymatous tissues which had been crushed both on the inside of the callus pad and in the zone where the two callus pads met. This provided a continuous bridge between the buried infection and the canker on the surface. As noted above, the canker on the surface appeared to be of relatively recent origin. This general arrangement of the tissues is characteristic of latent cankers, though the effect is naturally more striking in the wounds which are most nearly healed. It has also been found that the fibres in the base of the pruned branch are normally heavily infected, and that both intra- and intercellular hyphae occur. This can be seen clearly in Pl. 12, figs. 4 and 5, both of which illustrate cankers of this type.

The distribution of the tissues described above suggests that the course of infection is roughly as follows. It would appear that the original infection was either present in fibres at the base of the shoot below the pruning cut at the time of pruning, or that infection occurred soon afterwards, and was confined to the fibres. Of these alternatives the former seems the more probable, since it is difficult to understand why a new infection should be confined to the fibres, while it is relatively easy to understand the removal of a water-shoot with an infection near its base during pruning leaving a fibre infection behind the pruning cut. Once established in the fibres one would expect further penetration of the pathogen to be severely restricted, both by the natural reactions of the host, and by its own weak growth within the fibres. As the wound heals, however, conditions change, since the crushed tissue zone on the inside of the callus pad may provide a more congenial medium for growth, and allow the pathogen to emerge from the fibres and become active again.

Once established within this crushed zone the pathogen is in a position to reinfect the callus pad when a suitable wound occurs, and this may be formed either through splitting of the rapidly growing callus tissues or in the crushed zone where the callus pads meet.

Discussion

When considering the foregoing description of the cankers on apple and their development, it must be remembered that the observations were confined to normal branches on vigorous trees. The final effect of a disease of this type depends on the extent to which the pathogen can spread within the host, and this is obviously influenced as much by the vigour of the host as by the vigour of the pathogen. A striking example of the extent to which a canker type of disease depends for its success on the condition of the host is afforded by the behaviour of *Calonectria rigidiuscula* (Berk. & Br.) Sacc. on cacao, where the damage caused may range from a small rapidly occluded lesion on a healthy tree to a virulent dieback which may finally result in the death of a weak tree (Crowdy, 1947). There is little doubt that the behaviour of *Nectria galligena* is essentially similar, though it seldom appears to be occluded so easily and neither does it seem able to cause such devastating damage. The importance of this balance in controlling the spread of the pathogen in the peripheral tissues is relatively obvious and, since the tissues are accessible, it has been possible to disturb the balance experimentally, either by weakening the pathogen by applying a fungicide or by stimulating the reaction of the host by applying a growth substance which will stimulate callus formation. The effect, a marked regeneration of the host tissues at the edge of the canker (Crowdy, 1948, 1949), is essentially the same in either case. In the early stages of callus formation no fibres are formed in the tissues, therefore the question of fibre spread does not arise when considering the early stages of recovery. The pathogen, however, may be spreading in the fibres at the edge of the callus tissues and may re-emerge behind the phellogen; the isolated phellogens formed along the fibre are evidence that this does, in fact, take place. As long as these points of re-emergence are occluded immediately, there is probably no harm done, but if the formation of phellogens ceases with callus activity during the dormant season, this spread may prove extremely important, and might make successful control by treatment with plant-growth substances impossible.

The vegetative vigour of the host is probably of equal importance in checking the spread of the pathogen in the xylem tissues, since it will control the rapidity and completeness of the host's wound reactions. The rather surprising failure of the pathogen to spread from the xylem fibres into the adjacent healthy tissues may be due in part to the ability of the host to confine any hyphae which show a tendency to escape, but it may also be due to the weakened state of the hyphae within the fibres which precludes the formation of a sufficiently high concentration of toxins to allow the penetration of healthy tissue.

Except possibly in the case of the infection of partially healed pruning cuts, it is

difficult to assess the importance of the spread of *N. galligena* in the xylem fibres, but it would appear that little attention need be paid to a pathogen which is growing too weakly to emerge from a tissue which was dead prior to infection and which it is unable to destroy. The presence of the pathogen in the fibres may, on the other hand, assume importance in special circumstances which would of course include any breakdown in the resistance of the host.

Control

While it is obviously not possible in the light of the evidence given above to suggest any definite measures for the control of the disease, it may be of interest to consider the implications of some of the findings. In the first place it appears that the spread of the lesion can be checked to some extent if the host is vigorous, and this has been demonstrated experimentally by checking the spread of cankers with plant-growth substances which are known to stimulate callusing, and is reflected by the inclusion in most recommendations for canker control of suggestions for maintaining the vigour of the host. Unfortunately, there is no evidence that treatment with plant-growth substances is able to check the spread of the pathogen in the fibres, and the success of the stimulated callus pad may in part be due to their absence and the check to spread may be only temporary. This treatment does, however, demonstrate that, if stimulated, the phellogen can be strong enough to stop spread. There is also some evidence that the spread can be checked if the pathogen is treated with a fungicide; in this case the balance is tipped in the other direction and gives the normal host a chance to check the development of the weakened pathogen. Up to the present the main recommendation for control of the disease has been to cut out and remove the diseased tissue, and when carrying out this treatment the fact that there may be appreciable spread beyond the visible limits of the lesion must be taken into consideration, and the cut must be made far enough away from the lesion to allow for this.

A further point of interest when considering control is the possibility that the infections on healing pruning cuts may have arisen from infections which have been established in the basal tissues of the pruned stem prior to its removal. With most wound dressings the properties demanded are a certain fungicidal value without excessive phytotoxicity, combined with the ability to form a durable covering to the cut. This type of dressing is, however, unlikely to affect a pathogen already established in the tissues, and it is interesting to speculate on the possible efficacy of a wound dressing which is chosen primarily for its fungicidal value and its ability to penetrate deeply into the host tissues, killing any pathogen already present and protecting the exposed cut with an appreciable belt of fungicide-treated wood. It would be necessary to examine carefully the effect of such treatment on the wound before making any general use of such a treatment.

In closing, the author would like to express his gratitude to Prof. Harris for permission to use the photographic equipment in the Zoology Department of the University of Bristol and to Mr Latham, of the Zoology Department, for taking the photographs. He would also like to thank Mr R. W. Marsh, of Long Ashton Research Station, for his advice and interest during the course of the work.

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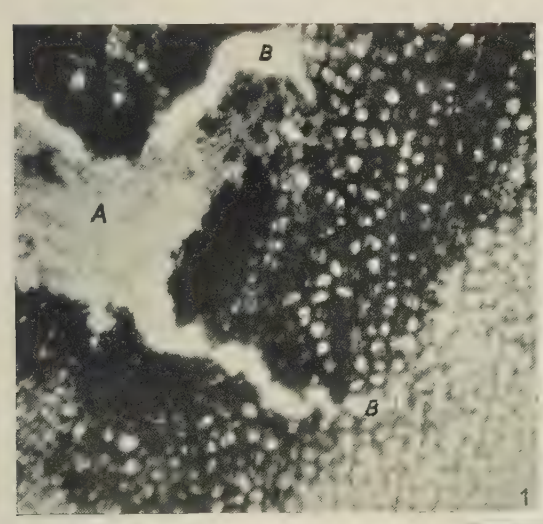

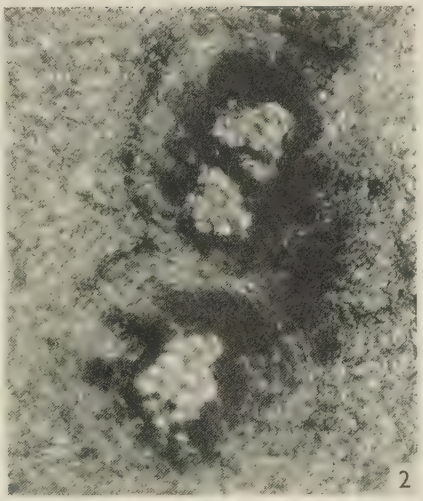

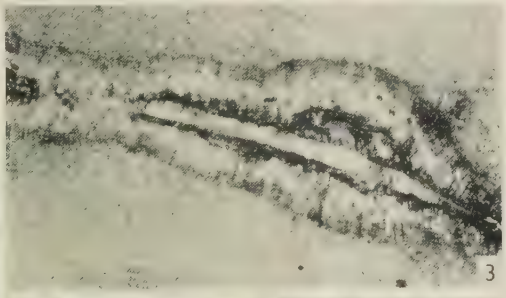

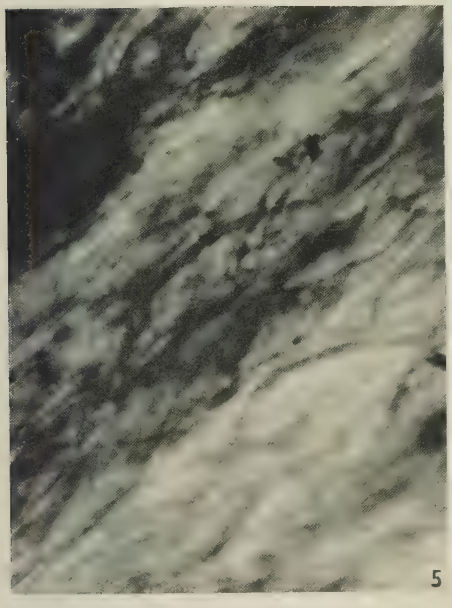

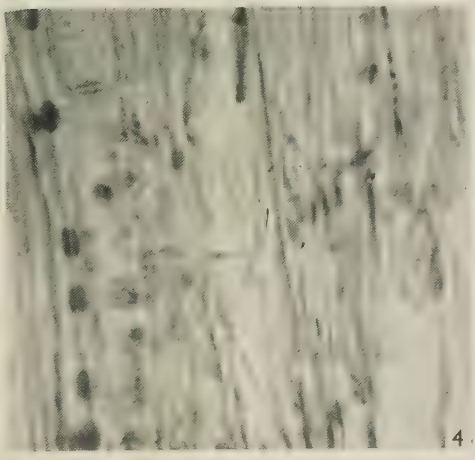

CROWDY—*Observations on apple canker*

EXPLANATION OF PLATE 12

Fig. 1. Transverse section at edge of canker showing aggregation of mycelium near wound barrier (*A*) and associated splits (*B*). (× 200.)

Fig. 2. Transverse section of peripheral tissues showing subsidiary wound barriers associated with fibre bundle. Worcester Pearmain. (× 200.)

Fig. 3. Longitudinal section of peripheral tissues showing subsidiary wound barriers associated with fibre bundle. Worcester Pearmain. (× 50.)

Fig. 4. Longitudinal section showing intracellular mycelium in xylem fibres associated with a latent infection. Cox's Orange Pippin. (× 600.)

Fig. 5. Longitudinal section showing intercellular mycelium in xylem fibres associated with a latent infection. Worcester Pearmain. (× 600.)

[*Footnote*]. Since going to press the author's attention has been drawn to a paper by J. C. Mooi, 'Kanker en Takinsterving van de Wilg veroorzaakt door *Nectria galligena* en *Cryptodiaporthe salicina*' (Baarn: Uitgeverij & Drukkerij Hollandia, 1948). In this paper Dr Mooi describes stages in the development of willow canker which are very similar to those found in apple.

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THE RELATION BETWEEN MOISTURE CONTENT AND MOULDING IN CURED HAY

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(With 1 Text-figure)

Samples of cured hay have been stored in closed containers at relative humidities of 0–95 % at temperatures varying from 5 to 25° C. A curve relating equilibrium moisture content to relative humidity of the store has been constructed and the incidence of first mould growth noted. An explanation has been suggested to account for the apparent discrepancy between earlier published work concerning the onset of moulding and conditions normally prevailing on the farm. In laboratory storage experiments temperature conditions are relatively constant, whereas with hay stacked in the open air the high atmospheric humidity in winter is offset by a lowered temperature, and the higher summer temperature is accompanied by a lower moisture content in the hay.

Introduction

During an investigation into the American method of hay finishing in the barn, it became necessary to know under what conditions of storage the hay could be considered 'safe', i.e. unlikely to mould or heat. The likelihood of moulding has always been regarded primarily as a function of the moisture content of the material and the relative humidity of the surrounding air, and for some time past it has been recognized that the equilibrium moisture content of a substance is governed by the amount of moisture in the air. Results have been published for the equilibrium moisture contents at various relative humidities of such materials as bacon, meat, cereals and many animal feeding-stuffs, including hay. Whilst many workers (Galloway, 1935; Barton-Wright & Tomkins, 1940; Milner & Geddes, 1946) regard 75% as the maximum relative humidity permissible for a wide variety of substances if moulding is to be prevented, Dexter (1947) and Dexter, Sheldon & Rose (1946) give 80–85% R.H. as the maximum for hay. On the other hand, Snow, Crichton & Wright (1944*b*) following Wright's (1941) earlier work on dried grass meal, state that over long periods 63% R.H., and for short periods 69% R.H., are the most that can be allowed. Both these latter groups of workers obtained the same general relationship between the moisture content and the relative humidity of hay, so that the difference is mainly one of interpretation.

Hay stacks in all parts of the British Isles stand exposed to winter weather, when the relative humidity is rarely below 80% and often nearer 90% for at least 4 months, and yet little or no moulding occurs provided the hay was properly cured in the first place. There thus appeared to be a marked discrepancy between the laboratory

results and those obtained in normal farm practice. The work described in this paper was designed to ascertain the reason for this discrepancy.

As a preliminary step it was essential to know what moisture content hay stored in outside stacks reaches towards the end of the wettest part of the year. For this purpose twenty-one stacks made in June and July were sampled in February at a depth of 2 ft. from the outside edge, usually at a height of 4–5 ft. The results were surprisingly uniform. The average moisture content was 17%, and two-thirds of the samples fell within the group 16–18%; the maximum deviation from the average was ±3%. From the curves given by Dexter *et al.* (1946) and Snow *et al.* (1944*b*) it was found that 17% of moisture corresponded to approximately 80% R.H., yet the latter authors had found mould mycelium development on several feeding-stuffs after prolonged storage at relative humidities as low as 67% and fairly quickly (3 months) at 70%. In view of this contradictory evidence it seemed desirable to repeat briefly the storage tests on hay at different humidities in order to check the relation between moisture content and moulding.

Experimental methods

As in the work of Snow *et al.* (1944*b*), large shallow glass vessels were used to store the hay over solutions of sulphuric acid calculated to provide relative humidities of 0, 20, 60, 65, 70, 75, 80, 82, 84, 86, 88, 90 and 95%. The hay (2 g.) was placed on open Petri dishes and weighed at regular intervals; at each weighing the dishes were covered with a tared cover-glass and inspected with a low-power microscope. The moisture content of the bulk hay sample was determined at the beginning of the experiment, and of the individual samples at the end of the storage period by drying in an air oven at 102° C. for 3 hr. From a knowledge of the weight of dry matter in each sample at the beginning, an approximate moisture content could be calculated for any sample at the first appearance of mould. Any loss of dry matter during storage would invalidate the calculation of moisture content both at equilibrium and at first moulding, but preliminary trials showed that, although such losses were considerable when fresh grass was so stored, they were negligible for hay. Comparison of the measured dry weight of the hay at the end of the experiment with the dry weight calculated from the moisture content of the sample at the commencement showed that loss of dry matter only occurred at 85, 90 and 95% R.H. to an extent of 1, 2 and 4% respectively of the original dry weights.

In the experiments of Snow *et al.* (1944*b*) the hay was used in the form of a meal. In the present work the first series of experiments was designed to establish whether the physical form of the hay, i.e. whether the hay was in long or short lengths or ground to a meal, had any effect on the moisture content or mould growth. The hay was separated into its four main constituent grasses and each stored separately in uncut lengths of 26–30 in. (approx. 10 g.) wound on to a wire frame, in 2 and ½ in. lengths and also as a meal after grinding in a hammer mill.

These samples were all stored in the laboratory, where the temperature was relatively constant at $19 \pm 2°$ C. This is far higher than in a stack exposed to the outside atmosphere and it seemed likely that such artificial conditions might considerably encourage mould growth. Accordingly, a second series of samples was placed in humidity chambers in the open air during the period January to March when the average outside air temperature was about 5° C. In this series the long uncut lengths were dispensed with and the humidity range reduced to 69–95%. The results showed the striking effect of temperature on mould growth. A third series was therefore set up in which the effect of temperature was studied under more controlled conditions. Hay in $\frac{1}{2}$ in. lengths was stored in atmospheres of 75 and 85% R.H. at 5, 15, 20 and 25° C. At the same time, beer-wort agar plates inoculated with mould spores were stored at 5, 15, 25 and 30° C. to demonstrate the effect of temperature on mould growth on a medium uncomplicated by such factors as the physical form and structure of the hay.

RESULTS

Series 1

Moisture. The effect of species differences on moisture content was negligible, only the meadow grass (*Poa pratensis*) being appreciably lower than the mean. This may be because meadow grass is generally more mature than the more important species such as timothy (*Phleum pratense*) or cocksfoot (*Dactylis glomerata*) when the grass is cut, which would agree with Dexter's (1947) finding that the moisture content of hay made from mature grass was lower than that of hay from young grass at any given relative humidity. Similarly, the size of the hay (i.e. whether uncut, cut or ground) was without influence on the amount of water absorbed at a given humidity. The only difference caused by the size of the hay was in the length of time taken to reach moisture equilibrium. The ground meal and $\frac{1}{2}$ in. lengths usually attained a constant weight 1–2 days before the longer samples. Table 1 illustrates these points for the hay samples stored at 60% R.H. A short trial was

TABLE 1. *The effect of botanical species and size of sample on moisture content* (%) *at equilibrium with an atmosphere of* 60% R.H.

	Size of sample				
Botanical species	Uncut	2 in.	$\frac{1}{2}$ in.	Meal	Mean
Timothy (*Phleum pratense*)	10·6	11·1	10·8	10·3	10·7
Meadow grass (*Poa pratensis*)	10·2	9·9	10·1	9·6	10·0
Yorkshire fog (*Holcus lanatus*)	10·4	10·9	10·4	10·9	10·6
Italian rye (*Lolium italicum*)	10·8	10·4	11·2	10·8	10·6
Mean	10·5	10·6	10·6	10·4	10·5

made to establish first whether the moisture content of the hay at the start of a storage experiment or the weight of material taken influenced the final moisture

content. Table 2 gives the moisture contents of six samples of hay, cut to $\frac{1}{2}$ in. lengths, stored at 90% R.H. and 19° C. Three of the samples weighing respectively 0·5, 2·0 and 4·0 g. were taken from one-half of a bulk sample of hay and the other three from the other half. Each half of the bulk sample had been allowed to attain a different moisture content before storage began. The 4 g. samples took rather longer to attain a steady value, but the original moisture content did not appear to influence the value reached. In all subsequent work samples weighing 2 g. and cut

TABLE 2. *The effect of original moisture content and size of sample on equilibrium moisture content*

Time stored at 90% R.H. (days)	Moisture content (%)					
	Wt. of sample (g.)			Wt. of sample (g.)		
	$\frac{1}{2}$	2	4	$\frac{1}{2}$	2	4
0	8·1	8·1	8·1	13·1	13·1	13·1
1	19·0	17·9	16·9	19·1	17·2	17·7
2	21·6	20·3	19·4	21·4	20·3	19·9
4	22·3	22·1	21·9	22·8	22·3	21·3
10	22·8	22·6	23·2	23·6	22·9	22·5

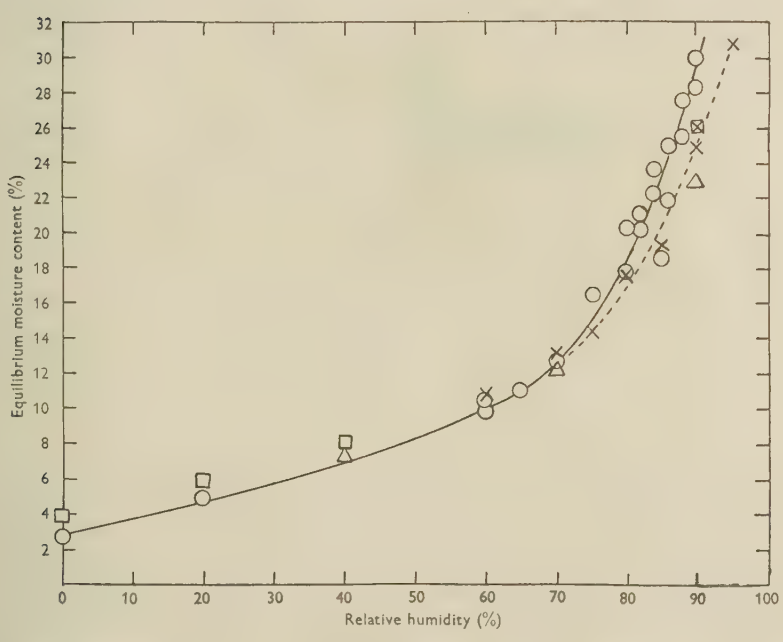

Fig. 1. The equilibrium moisture content of hay at various humidities. ○——○ Series 1, stored at 19° C.; ×– – –× Series 2, stored at 5° C.; △ Snow, Crichton & Wright (1944*b*); □ Dexter, Sheldon & Rose (1946).

to $\frac{1}{2}$–$\frac{1}{4}$ in. lengths were used. Fig. 1 records the mean equilibrium moisture contents of the sixteen samples obtained at the various storage humidities. Some replicate points have been included at the higher humidities, where errors arising from mould growth and loss of dry matter caused some divergencies. In this figure data abstracted from the corresponding curves of Dexter *et al.* (1946), and Snow *et al.* (1944*b*) have been plotted for comparison. Considering the differences in technique and original material, the three sets of results shown in Fig. 1 agree well.

Mould growth. Mould growth occurred on hay stored at 70% R.H. and at higher humidities; at 65% R.H. or lower humidities no mould was visible after 14 months' storage when examined at a magnification of ×64. Growth was first seen as thin strands of mycelium which later developed sporing heads, as described and illustrated by Wright (1941). It was noticeable that very frequently the first growth occurred at the cut ends of the pieces of hay, presumably where nutrient material was readily available and where the hyphae did not need to penetrate the smooth outer layer of the hay. Similarly, nodes, at which the smooth stem is interrupted by a joint, often developed a strong growth of mycelia more quickly than other parts of the stems. Table 3 records the approximate moisture content at first moulding, and the time of storage before mould was visible at some of the higher storage humidities. Plotting the logarithm of the period in days required to produce mould growth against relative humidity gave a smooth curve similar to those shown by Snow *et al.* (1944*a*).

TABLE 3. *Incidence of mould growth during storage at* 19–20° C.

Relative humidity (%)	Moisture content at moulding (%)	Days to develop mould
70	12·9	*c.* 200
75	15·7	19
80	18·2	6
82	20·4	4
84	22·5	3
85	21·6	4
86	23·8	3
88	27·6	2
90	28·0	2

Series 2

As already noted, the humidity chambers in this experiment were placed in the open air during January to March, a period of 54 days, and a continuous trace of air temperatures made on a recording thermograph. Except on 3 days during the first 20, the temperature was between 0 and 4° C.; from the twentieth to the thirty-eighth day it was consistently between 4 and 10° C.; for the next week between 0 and 4° C.; and during the last 8 days between 2 and 12° C. The average temperature might fairly be taken as 5° C.

Moisture. The equilibrium moisture contents at humidities from 60 to 95% have

been plotted on Fig. 1 for comparison with those from Series 1. The differences can be ascribed entirely to the different hay used in the two experiments, since, as will be seen later (Table 4), a lowering of temperature caused a slight increase in equilibrium moisture content.

Mould growth. At the lower temperatures the rate of moulding was markedly different from that of the hay stored at similar humidities in the higher temperature of the laboratory. Because of the seasonal increase in outside air temperature the experiment was terminated after only 54 days, but in that time only the hay stored at 90 and 95% R.H. showed any visible mould growth. Moreover, at these two humidities the times taken to mould were 21 and 5 days respectively, and in both cases only slight amounts of mycelium had formed, whereas at the same humidities at 19° C. mould growth commenced after 2 days and fructifications were rapidly formed. Absence of fructification in dried grass stored at 18–20° C. was recorded by Wright (1941) only at relative humidities below 70%.

Series 3

In the first place mould spores derived from hay kept at 90% R.H. in the laboratory were suspended in sterile water and plated on a beer-wort agar.* The agar plates were then stored at 5, 15, 25 and 30° C.; the initial relative humidity can be assumed to have been 100%, although this would probably drop at all temperatures, but most rapidly at 25 and 30° C. Discrete colonies were visible on the plates at 25 and 30° C. after 2 days, and on those at 15° C. after 4 days; the plates at 5° C. showed no sign of growth after 80 days. The great effect of temperature on the growth of moulds from hay even on a medium with a high moisture content was thus clearly demonstrated.

From the Air Ministry Meteorological Office tables of averages of humidities for the British Isles and the *Monthly Weather Reports* (M.O. 503), good approximations to the average monthly relative humidity and dry-bulb temperature can be obtained. If the inclusive period May to September is called 'summer' and October to April called 'winter' it can be calculated† that fair averages would be 15° C. and 73% R.H. for the summer months and 6° C. with 82% R.H. for the winter months. To simulate these conditions, relative humidity chambers at 75 and 85% were stored at 5, 15, 20 and 25° C. Hay (2 g.), cut to $\frac{1}{2}$ in. size, was stored in each chamber and weighed and examined at intervals. Table 4 gives the results of the examinations for mould growth and equilibrium moisture contents at the various temperatures.

The observations from these two tests confirm the theory that the moulds normally present on hay will not rapidly grow at low temperatures even in atmospheres of fairly high relative humidity, nor are they likely to flourish at higher

* The suspension and plates were prepared by Dr C. Higginbottom.

† Taken from the records of Dalwhinnine, Aberdeen, Glasgow, Eskdalmuir, Church Fenton, Harrogate, Oxford, Edgbaston, Gorleston, South Farnborough, Eastbourne and Portland Bill.

temperatures up to about 15° C. provided the relative humidity is not much greater than 75%.

TABLE 4. *The effect of temperature on mould growth and absolute humidity at* 75 *and* 85% R.H.

	75% R.H.		85% R.H.	
Temp. of storage (° C.)	Mould growth	Equilibrium moisture content (%)	Mould growth	Equilibrium moisture content (%)
6	None up to 200 days	15·4	Growth after 120 days	22·1
15	None up to 200 days	15·0	Growth after 2 days	20·3
20	Growth after 6 days	15·0	Growth after 2 days	20·0
25	Growth after 28 days	14·8	Growth after 2 days	20·2

DISCUSSION

With a view to interpreting these results in relation to farm practice it is necessary to compare the conditions under which previous laboratory results have been obtained and the naturally occurring meteorological conditions of this country. The results of Wright (1941) and Snow *et al.* (1944*a*, *b*) were all obtained at temperatures of 18–20° C. Dexter *et al.* (1946) make no mention of the temperature of their storage experiments, but the inference is that these were made in the laboratory probably at 70° F. (21° C.). Thus the previously published data for the moulding of hay applies to samples stored some 5° C. above average summer and some 15° C. above average winter conditions. As the present results have demonstrated for hay and as Barton-Wright & Tomkins (1940) showed for flour, temperature has a considerable effect on mould growth, the lower the temperature the longer moulds take to establish themselves and the higher the moisture content allowable before moulding begins. On the other hand, temperature has only a small effect on the equilibrium moisture content at any given relative humidity.

It seems, therefore, that two conditions at least must be fulfilled before moulds will grow readily on cured hay. The first is that the temperature of the environment should be high enough to encourage mould growth, and the second is that the mould spores should absorb sufficient moisture to allow growth processes to commence. It is possible that when the temperature is favourable the moisture content of the spores themselves, as opposed to the substrate to which they adhere, determines the commencement of growth, and that the moisture content of the substrate governs the extent of subsequent growth. The critical moisture content for hay varies slightly with the storage temperature and appears to be about 15–16% in summer and about 18% in winter.

Conclusions

1. The chief factors governing mould growth on cured hay are the temperature at which it is stored and the relative humidity of the storage atmosphere.

2. The moisture content of the hay is a function of the relative humidity of the storage atmosphere, and it is not greatly affected by temperature. Hay containing less than 15–16% moisture in summer and 18% moisture in winter is unlikely to mould.

3. The conditions of temperature and relative humidity in the open stackyard in Britain are not favourable to mould growth on well-cured hay.

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THE INHIBITION OF HATCHING OF POTATO ROOT EELWORM (*HETERODERA ROSTOCHIENSIS* WOLL.) IN PARTIALLY STERILIZED SOIL

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Experiments on the hatching of *Heterodera rostochiensis* have shown that the addition of ammonium carbonate to potato root water markedly inhibits hatching when the concentration of ammonia introduced is approximately 100 p.p.m. The strong acid salts of ammonia in equivalent amounts have no such inhibitory effect.

These observations are linked with experiments on the effect of partial sterilization of soil on the hatching of *H. rostochiensis*, and it is demonstrated that delay of hatching in such soils is only effective so long as the ammonia concentration within the soil is maintained at a sufficiently high level.

In a number of experiments Cheal (1929), and Buckhurst & Fryer (1931) showed that potato plants grown in soil partially sterilized by steam grew well in spite of the addition to the soil of cysts of *Heterodera rostochiensis* Woll. in numbers that would normally have led to severe injury to the plants grown in unsterilized soil. When the same soil was replanted the following year without further treatment the plants failed. The inhibitory effect was temporary, and Buckhurst & Fryer (1931) concluded that 'potato sickness' was due to a combination of root eelworm infestation and some unknown nutritional factor. Carroll & McMahon (1935) also showed that when potatoes were grown in partially sterilized soil in pots, aqueous leachates from these pots failed to stimulate hatching from cysts of *H. rostochiensis*, whereas comparable leachates from unsterilized pots stimulated hatching in the normal way. They further showed that this inhibitory effect on hatching became less pronounced as the period of time between sterilization and planting increased, until there was no effect when the soil was sterilized 6 months previously. The same writers after further work (1937) concluded that *H. rostochiensis* was the real cause of potato sickness, and that the failure of the eelworm to produce symptoms in potatoes grown in freshly partially sterilized soil was due to a delay in hatching caused by a factor that inhibits the hatching stimulus of potato root excretions and is produced as a direct result of the sterilization. They were not able to indicate what the character of this factor was likely to be.

The most constant feature associated with partial sterilization of soil appears to be, as first shown by Russell & Hutchinson (1909), the effect on the micro-flora of the soil causing a marked rise in ammonium nitrogen followed later by a fall as conversion to nitrate occurred. The problem was therefore investigated from the point of view of the relationship of the effect of ammonium nitrogen on the hatching stimulus of potato root excretions upon the eggs of *H. rostochiensis*.

Laboratory hatching experiments were designed to test the effect of various ammonium compounds on the hatching stimulus of potato root excretions on *H. rostochiensis*, and pot experiments were carried out to determine the effect of partial sterilization on (*a*) the development of potato sickness as measured by the growth of the plants and the yield of tubers; and (*b*) the ammonia content of the soil and of aqueous leachates of the soil, and the relationship between ammonia concentration, and the power of the root excretions in the soil and in the leachates to stimulate hatching.

Laboratory hatching experiments

Potato root water was obtained by leaching soil samples obtained from pots in which potatoes were in active growth. Preliminary tests had shown that it was possible to inhibit hatching from potato root eelworm cysts by the addition to the potato root water of relatively small quantities of ammonium carbonate or ammonium hydroxide when the technique similar to that of Carroll & MacMahon was employed. Yet, when the Fenwick (1943) modification of the Gemmell single-cyst technique was used, no conclusive results were obtained as is shown in Table 1. In this experiment twenty-five cysts were submitted singly to each treatment, but the experiment was abandoned after 8 days as no treatment prevented hatching.

Table 1. *The effect of ammonium salts on hatching, using the Fenwick single-cyst technique*

Solution	NH_3 concentration (p.p.m.)	Total larval hatch in 8 days	Larvae per effective cyst
P.R.W.*	—	2709	14·1
P.R.W. + ammonium carbonate	50	1440	8·5
P.R.W. + ammonium carbonate	100	1540	9·1
P.R.W. + ammonium carbonate	150	1581	9·4
P.R.W. + ammonium sulphate	50	1483	7·5
P.R.W. + ammonium sulphate	100	2718	14·7
P.R.W. + ammonium sulphate	150	2569	14·5

* Potato root water.

Qualitative tests showed that there was considerable loss of ammonia from the small open cells containing ammonium carbonate but not from those containing ammonium sulphate. Quantitative estimations (at intervals over 4 days) of ammonia in solutions stored in different kinds of container were made and the results are given in Table 2.

As a further check a small hatching test was carried out using single cysts enclosed with the appropriate solution in Durham tubes each firmly closed by a rubber bung. Ten cysts were submitted to each treatment for 20 days. The contents were removed by pipette at frequent intervals, the larvae counted and the cyst replaced in fresh solution. Table 3 gives the results.

When the loss of ammonia was prevented it was possible to prevent hatching by

the addition of ammonium carbonate to the potato root water. It was therefore decided to employ the method used by Carroll & McMahon and to place a relatively large number of cysts together in the solution under test.

TABLE 2. *Determination of ammonia in solutions stored in various kinds of container*

	Ammonium sulphate			Ammonium carbonate		
Type of container ...	Covered watch-glass	Fenwick cells	Corked tube	Covered watch-glass	Fenwick cells	Corked tube
Storage time (days)	NH_3 (p.p.m.)	NH_3 (p.p.m.)	NH_3 (p.p.m.)	NH_3 (p.p.m.)	NH_3 (p.p.m.)	NH_3 (p.p.m.)
0	230	230	230	90	90	90
1	230	210	220	80	50	90
4	230	230	230	70	10	90

TABLE 3. *The effect of ammonium carbonate and ammonium sulphate on hatching, using closed tubes*

	20-day experimental period			20-day post-experimental period cysts transferred to P.R.W.*	
Solution	NH_3 (p.p.m.)	Total hatch	Effective cysts	Total hatch	Effective cysts
P.R.W.	—	521	9	510	10
P.R.W. + ammonium carbonate	100	0	0	124	3
P.R.W. + ammonium carbonate	150	0	0	210	6
P.R.W. + ammonium sulphate	150	459	9	555	10

* Potato root water.

The ammonia content of each mixture after making up was checked by the B.D.H. Nesslerizer, and the pH in Exps. 2 and 3 was determined electrometrically. The hatched larvae were counted every day or every other day when the solutions were replaced from the original stock. When the numbers were small all the larvae were counted, but as the numbers increased they were estimated from the number of larvae in a 0·5 ml. aliquot pipetted from a 10 ml. suspension of the larvae hatched. In all three experiments the number of larvae left in the cysts in each batch after the hatching tests had finished was similarly estimated, but the cysts had first to be crushed in a small quantity of water.

Experiment 1

One hundred cysts were placed in each of three solutions, viz. potato root water, potato root water with ammonium carbonate added to give 100 p.p.m. of ammonia, potato root water containing ammonium sulphate to give the same concentration of ammonia. The results are given in Table 4.

TABLE 4. *The effect of ammonium carbonate and ammonium sulphate on hatching*

Solution	NH_3 content (p.p.m.)	Larvae hatched in 12 days	Larvae left in cysts
P.R.W.*	—	23,020	14,250
P.R.W. + ammonium carbonate	100	2	34,000
P.R.W. + ammonium sulphate	100	18,480	15,500

* Potato root water.

Experiment 2

In this experiment the effect of three strengths of ammonia in the form of ammonium carbonate on the hatching stimulus of potato root water on fifty cysts was compared. The pH of each solution was determined, and after the experimental period of 7 days all the batches of cysts were placed in potato root water as a check on the viability of the cysts, and finally the number of larvae left in the cysts in each case was estimated by the method already described.

TABLE 5. *The effect of concentration of ammonium carbonate on hatching*

Solution	NH_3 content (p.p.m.)	pH	Estimated larval content of cysts	Larvae hatched in 7 days	Hatch as % of total larvae in cysts	Hatch in potato root water in 5 days
P.R.W.*	5	6·6	10,856	3,196	29·4	1,660
P.R.W. + ammonium carbonate	160	8·2	11,037	317	2·8	4,540
P.R.W. + ammonium carbonate	80	7·8	12,499	1,389	11·1	4,530
P.R.W. + ammonium carbonate	40	7·6	11,481	3,481	30·3	1,080

* Potato root water.

Experiment 3

A similar experiment was conducted with a wider range of ammonium compounds. The experimental period was extended to 17 days followed by 7 days in which all the cysts were placed in potato root water which was the same as that used in Exp. 2, diluted with an equal volume of water. Table 6 sets forth the results.

TABLE 6. *The effect of various ammonium salts on hatching*

Solution	NH_3 content (p.p.m.)	pH	Estimated larval content of cysts	Larvae hatched in 17 days	Hatch as % of total larvae in cysts	Hatch in potato root water in 7 days
P.R.W.*	—	6·67	9,736	2,597	26·6	808
P.R.W. + ammonium carbonate	100	8·25	6,331	71	1·1	2,700
P.R.W. + ammonium sulphate	105	6·46	9,011	1,871	20·7	1,200
P.R.W. + ammonium nitrate	115	6·61	13,596	3,956	29·1	1,500
P.R.W. + ammonium chloride	95	6·83	9,878	3,238	32·7	2,000
P.R.W. + ammonium acetate	100	6·88	10,001	361	3·6	1,700

* Potato root water.

The results show that hatching can be virtually prevented by the addition of ammonium carbonate to the potato root water, but that the addition of the ammonium salts of the strong mineral acids to give the same concentration of ammonia fails to inhibit hatching. The apparent inhibitory effect of ammonium acetate shown in Table 6 may be due to the direct effect of the acetate radicle. The pH figures included in Tables 5 and 6 indicate that as the pH of the ammonium carbonate mixture approaches 8·0 the hatching power of the potato root water diminishes and is almost inhibited at pH 8·2.

Pot experiments

(*a*) *To test the effect of partial sterilization of the soil on the hatching of* Heterodera rostochiensis *and on the development of potato sickness*

The soil used was a medium loam to which organic matter in the form of neutral peat had been added the previous season. Lime to give a pH of 7 was added. Eight pots were used, four of which were partially sterilized by heat in the autoclave. To two sterilized pots and two unsterilized pots were added 10,000 viable cysts—representing approximately two cysts/c.c. of soil. A dressing of mixed fertilizer was applied to each pot, which was planted with a sprouted tuber of the variety Sharpe's Express on 2 May. The pots were then plunged in soil.

The unsterilized infected pots produced plants with the usual symptoms associated with root-eelworm attack and died off prematurely. The rest of the plants remained apparently healthy, although the eelworm-free sterilized pots produced the best plants as judged by the foliage. No difference in the tops could be detected between sterilized infected pots and the unsterilized eelworm-free pots.

The tubers from each pot were weighed, and the yields given in Table 7 follow closely the overground appearance of the plants during the growing season.

Table 7. *The inhibitory effect of partially sterilized soil on the development of potato sickness as measured by yield of tubers*

Pot no.	Soil treatment	Yield of tubers in oz.
1	Sterilized	7
2	Sterilized	6·5
3	Sterilized and infected	4·25
4	Sterilized and infected	4·0
5	Unsterilized	4·5
6	Unsterilized	5·5
7	Unsterilized and infected	1·0
8	Unsterilized and infected	0·75

(*b*) *The effect of partial sterilization on the ammonia content of the soil and on hatching of* Heterodera rostochiensis *in the soil and in aqueous leachates of the soil*

During the course of the experiment just described the ammonia content of the soil was determined at about weekly intervals. Composite soil samples were taken

from the four sterilized pots and from the four unsterilized soils, and the ammonia content was determined on each sample by Richardson's (1938) modification of Olsen's method. At the same time 200 g. of each bulk sample were used to prepare an aqueous extract by adding 75 ml. of water, thoroughly mixing and filtering. The ammonia content of the leachate was determined by B.D.H. Nesslerizer.

The hatching experiments were begun 3 weeks after the pot experiment was set up. One hundred cysts were immersed in each of the two aqueous extracts from the sterilized and unsterilized soils respectively, the extracts being obtained at about weekly intervals so that the cysts were submitted to a new extract each time the ammonia determinations on the soil were made.

Batches of twenty cysts were placed in the appropriate extract in a glass capsule with lid and rendered airtight with the aid of vaseline. The larvae were all counted at first, but as the numbers increased the total number hatched was estimated by counting the larvae in a 0·5 ml. aliquot of a 10 ml. suspension of the larvae from which the total could be calculated. The solution was renewed and counts made every 2 days. The results of the ammonia determinations on the soil and on the extracts are given in Table 8.

The number of larvae hatched during the experimental period and the number of larvae left in the cysts and therefore the total potential number of larvae in the cysts at the beginning of the experiment are shown in Table 9.

TABLE 8. *Weekly ammonia determination on sterilized and unsterilized soils. Ammonia content in p.p.m. of soils and their aqueous extracts*

Time in days ...	7	14	21	28	35	42	49
Sterilized soil	—	175	295	440	192	62	28
Unsterilized soil	—	49	20	70	40	25	20
Extract from sterilized soil	—	—	115	120	35	15	8
Extract from unsterilized soil	—	—	16	12	6	3	4

TABLE 9. *Hatching in aqueous extracts of sterilized and unsterilized soil*

Time in days ...		7	14	21	28	Larvae left in cysts	Total larvae	Hatch as % of total larvae in cysts
Sterilized:	Larvae	0	29	5,389	12,809			
	NH_3	120	35	12	8	17,600	30,409	42·1
Unsterilized:	Larvae	12	4,652	19,432	28,012			
	NH_3	12	6	3	4	4,707	32,719	85·6

The results of the hatching in aqueous extracts are comparable with those obtained by Carroll & McMahon. Hatching in the extract from the unsterilized soil commenced during the first week of the hatching experiment, and the larvae hatched amounted to 4,652 by the fourteenth day and continued to increase rapidly to the twenty-eighth day when counting ceased.

In the extract from the sterilized soil, however, the onset of hatching was delayed a week, and the final figures show that the rate of hatching was about half that in the control. The concentration of ammonia fell rapidly in the sterilized series during the second week of the hatching experiment, and from the end of that week hatching speeded up.

Since the ammonia content of the aqueous extracts was always much lower than that of the soil itself, an attempt was made to gain indirect evidence of the rate of hatching in the soil in the pot experiment. When the ammonia concentration in the sterilized pots showed a decided fall from the peak figure of 440 p.p.m. to 192 p.p.m., the larval population of a representative sample of the cysts in the soil in both sterilized and unsterilized pots was estimated. As in the previous experiments soil extracts were prepared by filtration of a mixture of 75 ml. of water and 200 g. of soil. Fifty cysts were collected at random from each lot of soil left after filtration, and the larval content estimated as described earlier. The larvae in fifty cysts taken from the stock used for infecting the soil for the pot experiments were also counted. The results of the weekly counts are shown in Table 10 along with the ammonia content of the soil at the time of sampling.

TABLE 10. *Larval content of fifty cysts in sterilized and unsterilized infected soil during the growing season*

Time after planting in weeks	Non-sterilized soil		Sterilized soil	
	Larvae	NH_3 (p.p.m.)	Larvae	NH_3 (p.p.m.)
5	5,360	40	12,144	192
6	6,200	25	11,680	62
7	6,080	25	8,200	28
8	6,680	—	5,600	—
9	5,640	—	5,440	—

Larval content of fifty cysts taken from original stock, 14,000.

The results of these counts are interesting in conjunction with the ammonia figures for the sterilized soil. Little or no hatching evidently occurred during the first 5 or 6 weeks that elapsed from the time of planting in the sterilized soil, during which time the ammonia concentration rose to 440 p.p.m. and then began to decline. As the ammonia fell from 192 p.p.m. the number of larvae left in the cysts also decreased, i.e. the rate of hatching increased. The figures for the unsterilized soil, on the other hand, seem to indicate that hatching had reached its peak by the time the counting began, for the number of larvae left in the cysts remained more or less unchanged.

DISCUSSION

Evidence from the experiments described indicates that where the concentration of ammonia in a partially sterilized soil rises sufficiently high, then the root excretion of potatoes growing in that soil loses its power to stimulate hatching of *H. rostochiensis*. The laboratory hatching experiments using various ammonium compounds

showed that whereas ammonium carbonate at appropriate strengths inhibited the hatching stimulus of potato root water, the ammonium salts of the strong mineral acids, viz. sulphate, chloride and nitrate at equivalent strengths, failed to affect the rate of hatching. During the period of our investigations we were unaware of the nature of the hatching factor in potato root excretion, and we were inclined to the view that the inhibitory effect which we observed was directly associated with the presence of ammonia produced by aqueous hydrolysis of ammonium carbonate, such hydrolysis not occurring in strong acid salts of ammonia. However, Prof. A. R. Todd has kindly informed us that eclepic acid, which he has identified as the hatching factor, is very readily destroyed by alkalies, and has suggested that the results we have obtained are to be explained by simple alkali reaction.

Whilst we have no pH figures for the soil and aqueous leachates in the pot experiment described, from the results of experiments summarized in Tables 4 and 5 it seems clear that as the pH of the solution approaches 8 the inhibitory effect is evident and is almost complete at pH 8·2. These pH figures are attained in the ammonium carbonate solutions when the concentration is around 100 p.p.m. It must be remembered that heavy eelworm infestation can and does occur on soils, e.g. the warp lands in Yorkshire, where the natural pH approaches and even exceeds 8.

It is interesting to consider the conclusions of Buckhurst & Fryer (1931) and of Carroll & McMahon (1935, 1937) in the light of our results. Buckhurst & Fryer were impressed by the fact that although potatoes grew normally in freshly sterilized soil in spite of the addition of a very heavy cyst population, yet at lifting time the roots were heavily infested by cysts. Our own observations and those of Carroll & McMahon confirm this, and it is clear that in spite of the statement of Buckhurst & Fryer to the contrary, there may be a considerable delay in hatching in freshly partially sterilized soil. This delay, which is due to an effect on the root secretions, gives the plants time to become well established before hatching commences, and so enables them to grow well in spite of a heavy late eelworm infection. The delay in our pot experiment as indicated by examination of the cysts in the soil during the growing season was about 5 weeks. In the case of the first series of experiments of Carroll & McMahon the delay in soil sterilized just before planting, as judged by the hatching stimulus of aqueous soil leachates, was longer, significant numbers of larvae beginning to hatch after the lapse of 40 days. Since the ammonia concentration of the leachate is very considerably less than that of the soil, the delay in the pots themselves may have been longer still. In their second series of experiments Carroll & McMahon found that the retardation of hatching was apparent but not so long lived as in the earlier series. This kind of variation is only to be expected, since the factors that influence the activity of the soil flora, which is itself markedly affected by partial sterilization, e.g. available organic matter and temperature, are themselves variable. The fact that the delay in hatching occurs most markedly in the most recently sterilized soil in relation to time of planting is also to be expected

if, as we claim, the inhibitory factor is associated with the rise to a critical level of the soil ammonia due to biological activity in the soil. There is ample time for the ammonium nitrogen produced in soils sterilized some months before planting to be converted into nitrate before planting, so removing the inhibitory factor.

It seems evident, therefore, that the inhibitory effect on hatching produced in partially sterilized soil is due to a rise in ammonia as a result of the change in the soil flora, and this neutralizes the hatching-stimulating substance and hatching is retarded as long as the level of ammonia remains high enough.

The use of chemicals as dressings on infected soil as methods of preventing 'potato sickness' raises interesting questions in relation to the foregoing discussion. Edwards (1929) recorded the beneficial effects of naphthalene and carbon disulphide and to a lesser extent of calcium cyanide and calcium cyanamide when mixed with the soil prior to planting. He suggested that the good growth and yield in the plants may have been due to 'a partial sterilization effect'.

Russell & Hutchinson (1909) refer to the partial sterilizing effect of volatile antiseptics like carbon disulphide and toluene which, like heat treatment, cause a rise in ammonia in the soil, and Jacobs (1931) records that one of the effects of treating a cucumber soil with naphthalene was to cause a very considerable rise in the ammonia. It seems likely, therefore, that in some cases the beneficial effects on plant growth in eelworm-infested soil as a result of dressing the soil with chemical substances may be due to a true partial sterilization effect involving a rise in ammonia in the soil to a level sufficient to neutralize for a time the hatching-stimulating substance in the potato root water.

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OVERWINTERING OF APHIDS, ESPECIALLY *MYZUS PERSICAE* (SULZER), IN ROOT CLAMPS

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(With 3 Text-figures)

Mangold clamps in many districts of the British Isles were found to provide overwintering sites for *Myzus persicae* (Sulz.), *Hyperomyzus staphyleae* (Koch) and *Aulacorthum solani* (Kalt.). After a severe winter, when other means of overwintering are few, clamps may be the most important source of *Myzus persicae*. Only *Myzus ascalonicus* Doncaster was found in swede clamps.

Factors affecting the infestation of clamped mangolds by *M. persicae* were the number of aphids on the crop when lifted, the methods of topping and clamping the roots, and the temperature in the clamp. *M. persicae* was introduced on the leaves, and close topping was often an efficient means of control. Close topping did not control *Hyperomyzus staphyleae*; normally, this aphid does not seem to be a root-feeding species, but with artificially colonized mangolds it fed on both exposed roots and foliage. It is not known how this species enters the clamps. The temperature in clamps was influenced by that of the outside air and the type of cover, but changes were long-term and did not reflect diurnal variations in external air temperature. Straw, covered with soil, was the best form of cover.

In addition to harbouring *Myzus persicae*, mangold clamps are also important sources of sugar-beet yellows virus.

The extent to which crops become infested with *Myzus persicae* (Sulz.) in summer depends on many factors, such as the number that survive from the previous year, the weather during spring and summer and the incidence of predators and parasites. The numbers of early migrants, which appear to be particularly important in deciding the prevalence and importance of virus diseases in sugar-beet and potato crops, will depend on the numbers that have managed to overwinter. New overwintering habitats are still being discovered. The winter is passed in the form of eggs which appear to hatch and produce colonies only on certain *Prunus* spp., but in suitable conditions the aphid can continue through the winter as viviparae on various plants under glass and, if conditions are not too severe, out of doors on brassicae crops, sugar-beet stecklings, lettuce and certain weeds. In Britain there is no record of survival on clamped swedes, though a related species, *Myzus ascalonicus* Doncaster, frequently overwinters in this manner. Mangold clamps are common sources of *M. persicae* (Broadbent & Hull, 1947), and the present paper records observations and experiments on the various factors that influenced the infestation of clamps by this and certain other aphids.

SURVEYS

To gain information on the prevalence of aphids in clamps, surveys were made in various parts of England from 1946 to 1948. The infestation was estimated by examining twenty-five mangolds from the exposed face of the clamp; when aphids were too numerous to count, the proportion of roots infested was recorded. It was rare to find clamps in which a few roots were heavily infested while most were free, and, in general, a low percentage of roots infested indicated a small total population of aphids, and a high percentage infested indicated a large population. A similar method was used with experimental clamps, except that fifty mangolds were examined. During the spring of 1946 mangold and swede clamps were examined in several widely separated districts (Table 1), and, in addition, an intensive survey was made in an area of 12 sq. miles around Hackthorn, near Lincoln. Of the twenty-seven mangold clamps in this area, thirteen were infested, one lightly and twelve heavily. The species were not determined in every clamp, but both *M. persicae* and

TABLE 1. *Clamps inspected, February–May* 1946

	Mangold clamps													Swede clamps				
	M. persicae					*H. staphyleae*				*A. solani*				*M. ascalonicus*				
District	Total	o	+	++	+++	o	+	++	+++	o	+	++	+++	Total	o	+	++	+++
	Number of clamps																	
Derbyshire	8	7	1	o	o	8	o	o	o	8	o	o	o	13	2	4	3	4
Holland	10	3	1	2	4	6	1	2	1	8	2	o	o	o	.	.	.	.
Isle of Wight	1	o	o	o	1	1	o	o	o	1	o	o	o	o	.	.	.	.
Kesteven	3	o	o	1	2	1	o	1	1	3	o	o	o	o	.	.	.	.
Norfolk	1	o	o	o	1	o	o	o	1	1	o	o	o	o	.	.	.	.
Total	23	10	2	3	8	16	1	3	3	21	2	o	o	13	2	4	3	4

o = no aphids found. + + = 30–60 % roots infested.
+ = under 30 % roots infested. + + + = over 60 % roots infested.

TABLE 2. *Mangold clamps examined March–June* 1947

District	No. of clamps	No. of clamps infested with	
		M. persicae	*H. staphyleae*
Derbyshire	4	1	o
East Lothian	1	o	o
Hertfordshire	3	o	1
Holland	6	3	1
Isle of Ely	3	2	2
Kesteven	1	o	o
Lindsey	23	o	2
Norfolk	8	o	o
Nottinghamshire	2	o	o
Total	51	6	6

Hyperomyzus staphyleae were present. Of forty-nine clamps examined in all districts, twenty-seven were infested.

In 1947 *Myzus persicae* occurred in only six of the fifty-one clamps examined between March and June 1947 in various parts of the country, including the same area around Hackthorn surveyed the previous year.

In the spring of 1948 a larger survey was possible. Clamps in all sugar-beet growing areas were measured and examined during the first fortnight of April and again during the first fortnight of May. Samples of infested shoots were sent to the laboratory and the aphid species were noted. The data obtained in the various areas have been grouped into districts and summarized in Table 3. In the areas surveyed there was an average of 17·3 mangold crops/10 sq. miles; in early April the number of clamps was 8·5 and in May was 3·2/10 sq. miles; at both times about 60% of the clamps were infested. Infested clamps occurred in all districts, though few were found in Yorkshire and Scotland. The shoot samples were graded 1, 2 or 3 according to whether they were lightly, moderately or heavily infested, and the 'mean grading', which is the average of these numbers, is shown in Table 3.

Factors affecting aphid infestation

(1) *Infestation of the growing crop*

During October 1946, growing crops of mangolds and swedes were examined and the average number of *M. persicae* per plant was assessed from samples of 20–50 plants taken at random across each field. Except in the Holland district of Lincs, mangold crops were lightly infested, which probably explains, at least in part, the low populations in clamps in the spring of 1947 (Table 2). The only clamp which was heavily infested in 1947 (76% of the roots infested on 10 April) was made from the crop which was most heavily infested (sixty-two *M. persicae* per mangold). However, not all the heavily infested crops resulted in infested clamps.

The mangolds and swedes examined in Derbyshire were adjacent crops in the same field on each farm; *M. persicae* showed a preference for swedes, which had an average of 61·3 aphids per plant as compared with 1·5 aphids per plant on mangolds.

In 1947 aphid counts were made on three crops which were used for experimental clamps. The mean infestations per plant shortly before lifting were 14·1, 7·7 and 3·9; roots from these crops, clamped according to normal practice, were heavily infested in the following spring.

(2) *The aphid infestation on the roots at clamping time*

When aphids were first found in clamps their method of entry was unknown; it was assumed the *M. persicae* entered on the leaves on which they had been feeding. Most *M. persicae* were feeding on the outer leaves, but some were on the young leaves at the centre of the crown. Experimental clamps were made at various places in the autumns of 1946 and 1947 to determine if removing different amounts of leaf

TABLE 3. *Mangold clamps survey*, 1948

District	Area surveyed (sq.ml.)	No. of holdings growing mangolds (per 10 sq.ml.)	Clamps				Aphids (mean grading)*		
			April		May				
			No. clamps (per 10 sq.ml.)	Clamps infested (%)	No. clamps (per 10 sq.ml.)	Clamps infested (%)	No. of samples	*M. persicae*	*H. staphyleae*
Northern									
Scottish	66·5	7·8	3·1	14·3	1·1	0	3	0·0	0·0
Yorkshire, North Riding	51·5	23·7	9·9	0	2·7	0	0	—	—
Yorkshire, East Riding	32·5	14·8	7·7	16·0	1·8	16·7	3	1·0	1·3
Yorkshire, West Riding	31·0	21·9	9·4	0	1·9	0	0	—	—
East Midlands									
Lindsey	128·5	17·3	11·4	54·1	6·1	78·2	32	1·2	0·6
Kesteven	49·4	23·5	11·7	62·1	3·8	89·5	19	1·4	0·1
Holland	36·0	32·5	12·5	73·3	3·1	63·6	8	1·1	1·3
Nottinghamshire	35·5	32·7	12·1	44·2	3·9	42·9	6	1·7	0·2
Eastern									
Norfolk	183·7	18·9	6·6	70·2	2·4	75·0	32	1·7	0·2
East Suffolk	73·4	11·5	4·9	66·7	1·9	64·2	9	1·6	0·6
West Suffolk	56·2	9·4	4·6	57·7	2·0	63·6	8	1·5	0·5
Isle of Ely and Cambridgeshire	70·3	20·7	7·7	72·3	2·2	87·4	20	1·5	0·4
Huntingdonshire	27·5	26·2	16·7	97·8	6·9	94·7	13	0·9	0·9
Northamptonshire	27·0	25·2	13·7	83·8	3·3	100	11	0·6	0·6
Midlands									
Leicestershire and Warwickshire	14·0	40·7	22·1	3·2	12·9	44·4	1	1·0	0·0
Salop and Staffordshire	40·5	29·9	18·5	40·0	5·2	47·6	14	0·9	0·6
Herefordshire and Worcestershire	52·0	14·6	10·2	54·7	4·8	72·0	16	0·3	1·1
Home Counties									
Essex	68·5	6·4	2·9	20·0	1·2	75·0	2	2·0	0·0
Bedfordshire and Hertfordshire	41·0	8·8	4·1	58·8	0·7	0	6	1·3	0·3
South, south-west and Pembroke	141·5	11·0	7·9	50·9	3·5	74·0	11	0·8	0·5
Totals and means	1226·5	17·3	8·5	58·1	3·2	66·4	214	1·2	0·5

* *Mean grading*. The shoot samples were assessed 1, 2 or 3 according to whether they were lightly, moderately or heavily infested. The mean grading is the average of these numbers.

TABLE 4. *Aphids present on growing crops of mangolds and swedes—October* 1946

District	No. of fields	Mean no. of *M. persicae* per plant	Other aphids present
		Mangolds	
Derbyshire	4	1·5	*Aphis fabae*
Hertfordshire	7	0·1	*Aphis fabae, A. solani*
Holland	4	48·5	*Aphis fabae, A. solani, M. euphorbiae*
Isle of Ely	1	8·2	*Aphis fabae, A. solani, M. euphorbiae*
Kesteven	1	1·3	*Aphis fabae*
Lindsey	13	0·5	*Aphis fabae*
Norfolk	5	3·6	*Aphis fabae*
Nottinghamshire	1	1·0	*Aphis fabae*
		Swedes	
Derbyshire	4	61·3	*Brevicoryne brassicae*

TABLE 5. *Aphids in experimental clamps*

Site	Field infestation *M. persicae* per plant	Clamp treatment	Date examined	Roots infested (%)	Aphids present *M.p.*	*H.s.*	*A.s.*	*Roots rotten (%)
1946–47								
Isle of Ely	8·2	A	14 May	48	+	++	—	12
		B	14 May	5	+	—	—	8
		C	14 May	96	—	+++	—	3
Hackthorn (Lindsey)	0·4	A, B and C	April and May	0	—	—	—	—
Sprowston (Norfolk)	4·0	A, B and C	17 Mar. to 19 June	0	—	—	—	—
1947–48								
Isle of Ely	7·7	A	28 Apr.	100	++	+++	+++	10
		B	28 Apr.	100	+++	+++	++	8
		C	28 Apr.	72	+	+++	—	3
Hackthorn (Lindsey)	14·1	A	18 Mar.	36	+	++	—	—
		C	18 Mar.	4	—	+	—	—
Welton (Lindsey)	3·9	A	23 Mar.	34	+	+	—	2
		B	23 Mar.	48	—	++	—	24
		C	23 Mar.	54	—	++	—	64
Terrington (Norfolk)	4·6	A	24 Mar.		Clamp collapsed			100
		B	24 Mar.	52	+++	—	—	—
		C	24 Mar.	72	++	—	—	—

A = untopped.
B = 3–4 in. of crown left.
C = all leaves and shoots removed.
+, ++, +++, as Table 1.

M.p. = *Myzus persicae*.
H.s. = *Hyperomyzus staphyleae*.
A.s. = *Aulacorthum solani*.

* Isle of Ely records refer to rotten roots, Lindsey records to any lesions of fungal decay.

would affect the infestation in the clamp. For one clamp at each centre, the mangolds were clamped untopped; for another, they were chopped so that 3–4 in. of crown remained, usually with some outer leaves; for the third, all leaves and shoots were removed. The proportion of roots infested in the following spring is shown in

Table 5. The 1947 results were inconclusive; at Hackthorn and Sprowston no *M. persicae* was found in any of the clamps, however treated, and in the Isle of Ely there were aphids in all three clamps, but no *M. persicae* in the one containing roots with all leaves removed. The suggestion from this test that close topping was beneficial in controlling *M. persicae* was confirmed in 1948. Whereas there was no *M. persicae* in the clamps containing trimmed mangolds at Hackthorn or Welton in 1948, nor in the Isle of Ely clamp in 1947, this aphid was in the Isle of Ely and Terrington clamps in 1948. This may stress the need for extreme care in removing shoots if *M. persicae* is to be excluded. The roots for the Isle of Ely and Terrington clamps were topped in the field and some small shoots may have remained attached, whereas the Hackthorn and Welton clamps were made from roots first trimmed in the field and then carefully retrimmed at the clamping site. The results of these experiments showed that *M. persicae* are introduced on the leaves. Although the more green matter allowed in the clamp the greater is the danger of infestation, clamps of partially topped roots sometimes contain more aphids than clamps of untopped roots. This probably occurs because in a clamp of untopped roots the shoots are often wet and slimy. In the Isle of Ely clamps in 1947, roots in contact with rotting mangolds or leaves were usually free from aphids.

It appears that *M. persicae* can be controlled by removing all the leaves before clamping, but greater care would have to be taken than is normally possible in trimming commercial crops. Unless topping is complete, some aphids may survive on lateral buds and give rise to an infestation in the spring, as probably happened in the experiments at Ely and Terrington in 1947–8.

Hyperomyzus staphyleae was never seen on the tops of mangolds when they were examined in the field, and it was thought that this species, like some of its close relatives, might be a root-living form during the summer. The roots being clamped at the Isle of Ely site in 1947 were carefully examined; an average of 7·7 *Myzus persicae* per plant and an occasional *Aulacorthum solani* were found on the leaves of mangolds which had not been topped; a few *Myzus persicae* were seen crawling on the roots of plants which had been closely topped; but no *Hyperomyzus staphyleae* was seen on any plant, either on foliage or roots, yet this species was the most numerous when the clamps were opened in the following spring.

To gain further information on its habits, mangold plants in pots were infested with *H. staphyleae*. Heavy infestations developed on the leaves, on bud initials and on small lateral roots above soil level, but of ten infested plants examined, none had *H. staphlyeae* below soil level. The foliage was completely removed from six plants, and fifty *Myzus persicae* were placed on each of three roots and fifty *Hyperomyzus staphyleae* each on the other three. The following day the roots which had been colonized with *Myzus persicae* were aphid-free, whereas the *Hyperomyzus staphyleae* were feeding on the lateral rootlets of all three plants colonized. They continued to feed and breed there for 3–4 weeks, when the experiment was discontinued. Thus, although the origin of *H. staphyleae* infesting clamps remains undetermined,

individuals introduced into clamps on closely topped mangolds could live on the rootlets, whereas *Myzus persicae* could persist only when there were leaves present.

The opinion is widespread among farmers that closely topped roots will rot, but in the two Isle of Ely experiments with the variety New Century, closely topped mangolds kept better than the others. A variety like Kirsche's Ideal, in which the crown is large and diffuse, might suffer damage from close topping.

(3) *Temperatures within mangold clamps*

During the winter of 1946–7 temperatures inside mangold clamps were recorded, using either a vapour-pressure thermometer with a steel stem 4 ft. long or a mercury thermometer lowered into a hole made with an iron rod. Records were taken in Lincolnshire, Derbyshire and Hertfordshire; the means of all readings and the mean air temperatures during the winter and spring are shown in Fig. 1. The clamp

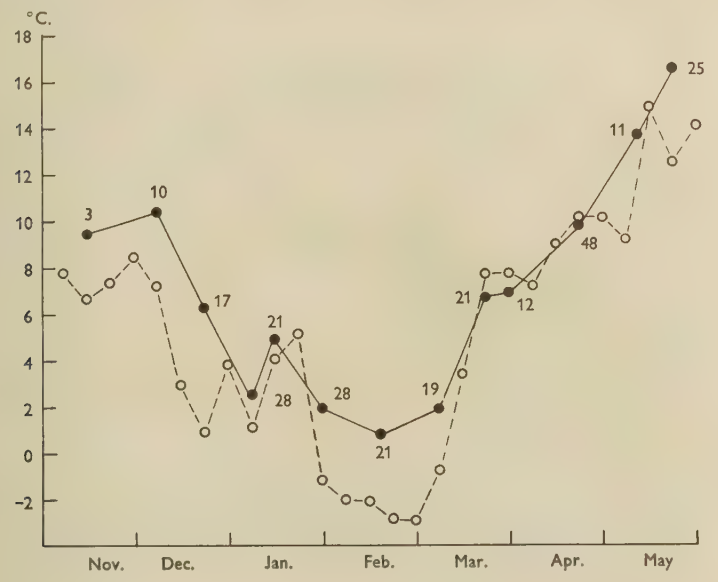

Fig. 1. Means of temperatures in mangold clamps and weekly mean air temperatures, 1946–7.

●——● clamp temperature, with number of readings; ○--○ air temperature.

temperatures were slightly higher than the mean air temperatures for most of the winter, and considerably higher during the prolonged period of frost in February. Three mercury-in-steel thermographs gave continuous temperature records for one clamp which was compared with that of a thermograph in a standard Stevenson screen near the clamp. The temperature within the clamp followed that of the

outside air, but fluctuations were smaller and diurnal variations in the external air temperature were not reflected inside the clamp. The outer mangolds in some clamps were injured by frost. During periods of prolonged frost, straw alone does not prevent loss unless the clamp is also covered by snow. The best-protected clamps were those covered with straw and soil, and damage by frost decreased with increasing depth of soil. The volume of the clamp did not affect the temperature within the limits 450–6000 cu.ft.

Internal temperatures of six experimental clamps (approximately $15 \times 6 \times 4$ ft. high) were taken with a vapour-pressure thermometer during 1947–8. Three clamps contained topped and three untopped mangolds. The clamps were made early in November and were half covered with soil at once, the soil cover being completed on 1 December. The temperatures in the clamps (Fig. 2) containing untopped

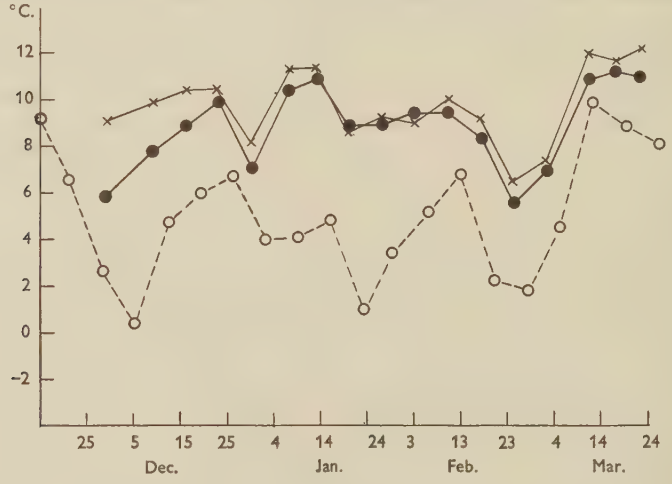

Fig. 2. Means of temperatures in mangold clamps and weekly mean air temperatures, 1947–8.

×——× Mean temperatures in three clamps of untopped mangolds.
●——● Mean temperatures in three clamps of trimmed mangolds.
○- - -○ Mean air temperature.

mangolds were at first higher than in those containing topped roots, but within a month the two were similar, both giving curves parallel, but at a higher level, to that of the mean air temperature.

Barker & Wallace (1946) obtained similar results in potato clamps. They also found a difference of 7–8° F. between the bottom of the clamp and the exposed side during a prolonged cold spell. A similar temperature gradient must have existed in the mangold clamps, for some of the outer mangolds were frozen, whereas the inner roots were not.

In the spring of 1947 clamp temperatures were below 5° C. for about 2 months, whereas in 1948 they were normally around 9° C. and never fell below 5° C. Aphids were more numerous in clamps in 1948 than in 1947 (Tables 2, 3), and it is probable that clamp temperatures, as well as the previous field infestations, influenced the degree of infestation. When clamp temperatures rise in spring the rate of aphid multiplication will increase; six of seven experimental clamps examined both in March and in April 1948 had larger infestations at the later date. In the extensive survey of 1948, 62% of infested clamps showed an increase in the percentage of roots infested between the beginning of April and the beginning of May, 30% showed a decline and 8% remained the same. The aphid population generally rises to a peak and then rapidly declines, either because of the migration of alatae or the depredations by parasites and predators (coccinellids, syrphids, *Aphidius* spp.) which often occur in clamps early in spring. The aphid population may increase later, particularly on the green mangold shoots which grow from the clamp cover in summer.

Dispersion of aphids and virus diseases from clamps

When alate aphids leave a mangold clamp they may fly to any nearby host plant and start an infestation. If the host plants are sugar beet, or other members of the same family, the migrating aphids may also carry virus diseases from the clamped mangolds to the field crop.

During 1946 healthy sugar-beet plants in pots were exposed in fields around Hackthorn for approximately 2-week periods; they were then returned to the glasshouse, examined for aphids and fumigated. Symptoms of diseases contracted during exposure were recorded as they appeared. Five plants were exposed at each site; the pots were sunk in the ground and watered when necessary. The sites were as follows:

(*a*) Within 2–3 yd. of mangold clamps. Clamp 1 contained about 3 tons of mangolds, no. 2 about ½ ton and no. 3 about 6 tons. In an effort to control the aphids in clamp 3, it was covered with loose straw into which benzene hexachloride dust was blown before the exposures started. The treatment did not kill the aphids; instead, the alatae swarmed out of the clamp in large numbers (Table 6).

(*b*) The headland of a beet-steckling field in spring and late summer.

(*c*) The headland of a sugar-beet seed crop.

(*d*) At distances of 4 ft. around yellows-infected sugar-beet plants in a barley crop and in a pea crop.

(*e*) Between a sugar-beet and a turnip crop.

(*f*) Where the stand of plants was thin in two sugar-beet fields.

(*g*) In a garden, 20 yd. from an insectary where aphids were cultured on yellows-infected sugar beet.

More of the plants exposed near mangold clamps became infected with yellows than at other sites (Table 6). The number of infections declined as the mangolds

became fewer, but infections occurred until early August near clamps 1 and 3. Of the plants near the clamps many fewer contracted mosaic than yellows; elsewhere mosaic was contracted only near the seed crop.

Sticky aphid traps (Broadbent, Doncaster, Hull & Watson 1948) were erected at most of the plant exposure sites and the catches of *Myzus persicae* and *Hyperomyzus staphyleae* are shown in Table 7. Catches of *Myzus persicae* were greater near clamps 2 and 3 in June than at the other sites. *Hyperomyzus staphyleae* was trapped only near mangold clamps.

TABLE 6. *Exposures of sugar-beet plants in pots*, 1946

Date exposed ...	21.v.	31.v.	13.vi.	29.vi.	9.vii.	25.vii.	8.viii.	20.viii.
Exposure site	No. of *M. persicae* on five plants							
Mangold clamp 1	2	4	1	3	9	14	1	5
Mangold clamp 2	4	1	23	0	0	—	—	—
Mangold clamp 3	—	—	252	12	43	6	3	—
No. of plants exposed at other sites	30	30	25	25	20	30	30	30
No. of aphids present	3	0	0	2	10	10	8	5
	No. of plants out of five infected with yellows							
Mangold clamp 1	0	2	5	3	1	0	2	0
Mangold clamp 2	3	3	2	2	1	—	—	—
Mangold clamp 3	—	—	5	5	5	5	3	—
No. of plants exposed at other sites	30	30	25	25	20	30	30	30
No. of plants infected with yellows	1	3	2	4	4	7	5	2
	No. of plants out of five infected with mosaic							
Mangold clamp 1	0	1	0	1	0	0	0	0
Mangold clamp 2	0	0	0	1	0	—	—	—
Mangold clamp 3	—	—	3	3	0	0	0	—
No. of plants exposed at other sites	30	30	25	25	20	30	30	30
No. of plants infected with mosaic	0	3	1	0	0	0	0	0

TABLE 7. *Aphids caught on sticky traps exposed near sugar-beet plants* (*Table* 6)

	M. persicae								*H. staphyleae*							
	June				July				June				July			
Week ...	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Near clamp 2	2	1	1	4	1	0	0	0	0	10	0	0	0	0	0	0
Near clamp 3	0	0	11	12	4	2	2	1	0	1	386	296	300	6	20	45
Mean catch at six other sites	0·2	0·2	0·3	0·7	1·5	0·2	0·2	0·2	0	0	0	0	0	0	0	0

In every sugar-beet field in the Hackthorn area surveyed for mangold clamps in 1946 and 1947, counts were made of aphids on ten pairs of plants and of virus in ten groups of 100 plants. There were too many mangold clamps in the area for any relation to be apparent between yellows infection in most sugar-beet crops and their proximity to mangold clamps. In 1946, however, the effect of one clamp was very evident. This contained 50 tons of mangolds, heavily infested with aphids, and remained *in situ* throughout the summer. Yellows appeared in an adjacent sugar-

beet crop in July, and the percentage of plants infected declined sharply with increasing distance from the clamp; at 200 yd. from the clamp infection was at the mean level for the district at that time. During the summer the disease spread and the results of counts in September in fields within 2 miles of the clamp are shown in Fig. 3. There is a significant relation between the number of aphids and yellows

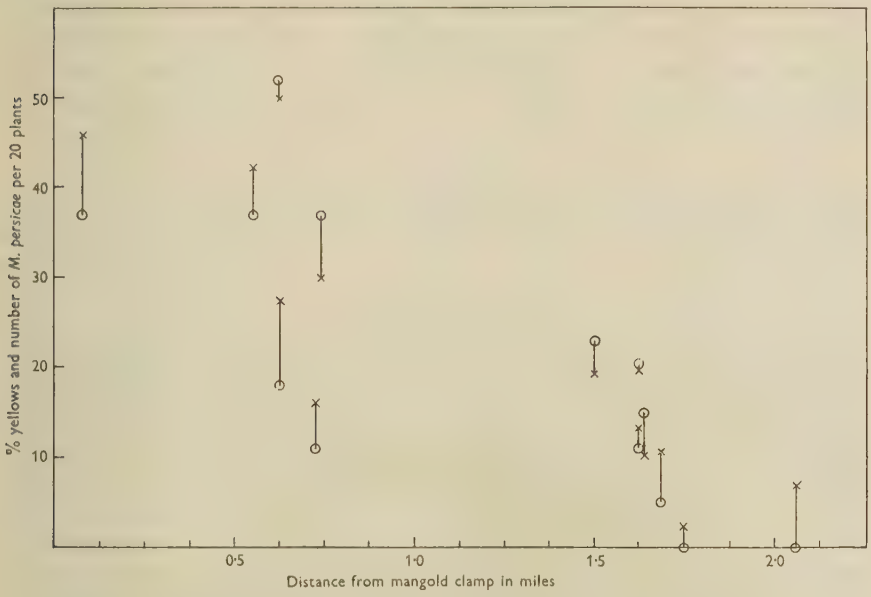

Fig. 3. The relation between numbers of *Myzus persicae* and yellows infection in sugar-beet fields and the distance from a mangold clamp.

× = yellows. ○ = *M. persicae.*

infection, and the numbers of aphids and infected plants both decrease with increasing distance from the clamp. It is obvious that this clamp was a major factor in determining both the population of *Myzus persicae* and virus infection in beet crops in its neighbourhood.

In July and August 1947 yellows-infected plants in most sugar-beet fields were confined to discrete patches. Plants near the centres of the patches were stunted and had clearly been infected since the end of May or early June. The plants in such patches carried colonies of *M. persicae*, but few or none occurred on the green plants. *Aphis fabae* occurred on most plants and caused severe injury to some, but these were distributed independently of the patches of yellows. These observations suggest that some winged *Myzus persicae* carrying yellows virus migrated to the fields in the spring, when they either infected single plants from which further

spread occurred by colonies of apterae developing from the progeny of these migrants, or they themselves infected several adjacent plants. As *M. persicae* was almost confined to the infected plants it seems that all the winged *M. persicae* initiating colonies in these beet crops in the spring or early summer were already infective and had therefore probably overwintered on diseased plants. The winter of 1946–7 was exceptionally severe; many winter crops were killed, and no surviving *M. persicae* was seen during inspections of beet-seed crops and winter brassicae in early spring. It is most probable that the aphids causing these early infections in sugar-beet fields came from mangold clamps. It is after such severe winters that clamps are most important compared with other sites of overwintering viviparae.

Control

Many farmers now sow root crops and plant potatoes earlier than in the past so that the time during which crops are exposed to direct infestation by aphids from mangold clamps has been increased. The time for which mangolds are kept in spring varies with local practices. In districts where mangolds are no longer required after early April, control of aphids is easily achieved by thoroughly clearing the clamp site; many of the clamps seen in 1948 contained only few roots, often being left to rot. On other farms considerable quantities of mangolds are retained in clamps for stock-feed until summer and for these special aphid control measures are desirable. Control measures would increase the cost of production of mangolds, and it is doubtful if most farmers would be willing to undertake them. Preliminary experiments with insecticides have not given promising results. Dusting with derris or 3% nicotine dust, at the rate of 1 lb. of dust/ton of mangolds, during clamping failed to control the aphid infestation. Some degree of control was obtained in June 1947 by injecting nicotine vapour into clamps through perforated tubes attached to a nicotine fumigation machine (Wilson, 1946). But before any insecticidal method can be recommended, the effect of the chemical on the roots requires investigating.

The authors wish to acknowledge the help of the agricultural staff of the British Sugar Corporation in making the survey in 1948; also the various farmers who gave facilities for the clamping experiments. Miss B. M. G. Hamlyn made the aphid trap identifications recorded in Table 6.

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COLORADO BEETLE IN THE CHANNEL ISLANDS, 1947 AND 1948

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(With 5 Text-figures)

Colorado beetles can survive up to 10 days in sea water and still be capable of flying when the temperature reaches 80° F. (26·67° C.). The beetles that reached the Channel Islands in May 1947 and in May 1948 prove that they can travel across about 30 miles of sea, the approximate distance of Guernsey from the Cherbourg peninsula. The invasions occurred as a result of migrant beetles from the Cherbourg peninsula which dropped on the sea between the Channel Islands and the French coast and drifted ashore.

Introduction

Since Riley (1877) first published his paper on the history of the spread and dispersion of the Colorado beetle, *Leptinotarsa decemlineata* (Say.) in America, little has been written concerning the natural spread of the beetle to new areas separated from infested zones by a wide expanse of sea. There are records, both in France and America, of the invasion of small islands near the coast of these countries—Belle-Ile, off the Brittany coast is an example—but in none are there any precise details of the way in which the beetles reached these islands. The island of Jersey, situated approximately 14 miles from the nearest French coast, is admirably suited for obtaining such information. Feytaud (1939*a*) mentioned reports of large falls of beetles on the sea between Brittany and Jersey, and warned the Channel Island authorities of the possibility of beetles arriving on the islands.

Except for an isolated colony discovered on 3 October 1939, Jersey remained free from infestation until 29 May 1943, when five isolated beetles were found in various localities near the coast on the south of the island. In 1944, however, four colonies were found on potato crops during July and August, which suggests that a number of the 1943 beetles had been overlooked. Owing to the German occupation, no strict control measures were possible until after the liberation in 1945, when vigorous measures were employed. These were almost completely successful by the end of 1946 (Small, 1947).

The 1947 invasion of Jersey

On 28 May a live beetle was found in a street in St Helier, and another on the beach near the town. On the following day fifty more beetles were found in various places, such as peoples' clothing, streets, lawns, sea walls, piers, bicycles, boats and gardens,

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and on 30 May the first beetle was found on potatoes. By 31 May the beetles had established themselves in considerable numbers on the potato crops, and had commenced laying eggs.

Between 28 May and 4 June, 389 live beetles were found, and during the whole season 648 beetles in all were recorded. Fig. 1 shows diagrammatically the course

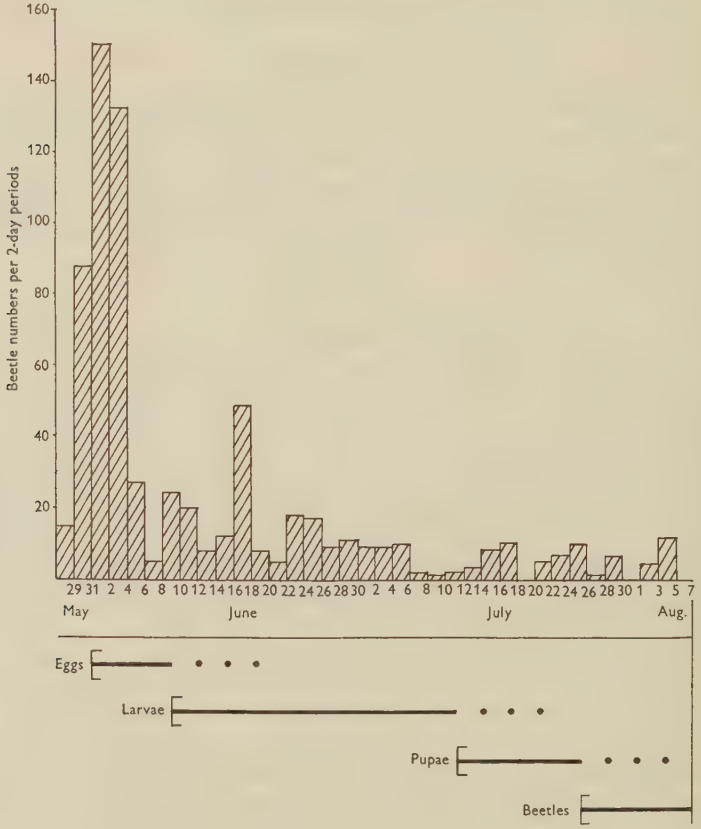

Fig. 1. Showing number of beetles recorded in 1947 invasion; also dates when eggs, grubs and beetles occurred

of the 1947 invasion and the speed with which the beetles commenced to lay eggs and establish themselves.

As reports came in, the positions of the beetles found were plotted on a map. During the first 2 days most of the beetles were found around St Helier, but by 30 May the majority of the reports came from the eastern side of the island. This dispelled the theory that the beetles arrived on boats from France as the apparent

concentration in the town at first suggested. By 4 June most of the beetles found were scattered along the eastern border of the island within 1–1½ miles of the coast, a form of distribution which did not suggest that they flew direct from France. A number of beaches were then searched, and on 6 June many dead beetles were found on the beach at Hâvre-des-pas, near the town, and varying numbers of dead beetles on beaches on the east, north, west and south with the largest numbers on the east and south. At Grève d'Azette on the south coast, counts were made over measured distances at different parts of the beach, which is approximately a mile in length, and 15,000–20,000 dead beetles were estimated to be scattered along the 33·5 ft. tide-mark. These beetles, found on 6 June, must have been there since

Fig. 2. Map showing the distribution scatter of beetles found in Jersey between 29 May and 6 June 1947.

○ Location of discoveries from 29 to 31 May. □ Location of discoveries from 1 to 3 June.
● Location of discoveries from 4 to 6 June.

4 June at least, since that was the date of the last 33·5 ft. tide. The dead beetles reported elsewhere were also found at the same tide-level. No live beetles were found on any of the beaches.

The 1947 invasion of Guernsey and Sark

On 29 May a live beetle was found in Guernsey on a hedge and another on a pile at the end of the jetty at St Peter Port. A third was found on 4 June on potatoes and a fourth between two rows of potatoes on the island of Sark. On 9 June, large numbers of dead beetles were found on the beaches of Guernsey, Sark and Herme well above the tide-mark of that day, indicating that they had been there for several

days, possibly since 4 June. Despite the large numbers of dead beetles on the beaches, only five live beetles were found on Guernsey and thirty-four on Sark during 1947.

Evidence suggesting a sea-borne invasion

There are several references to Colorado beetles surviving in sea water for a considerable period. Vayssière (1939) states that live Colorado beetles were washed up along the coast between La Baule and La Turballe in France. Feytaud (1936) also recorded heavy falls of beetles in the sea off the coast of France, and large numbers

Fig. 3. The Channel Islands showing position of the Ecrehos and Paternosters.

washed up on the beaches; he also mentioned that they can float for a considerable time, but did not say how long they can survive under such conditions.

Tests were made to determine how long beetles can survive in sea water. Ten beetles were placed in containers on 1 July. These were filled daily with fresh sea water which was agitated two or three times a day so that the beetles were immersed occasionally. On 3 July one beetle was dead. On 6 July six more had died, but three survived until 10 July. Some beetles may therefore be able to survive on a calm sea for 6–10 days. This test was repeated, and the beetles which had survived 6 days in the sea water were placed on seaweed under a bell jar in the sun. When the

temperature in the jar reached 80–85° F. (26·67–29·44° C.) they began to fly. When the temperature rose to 100° F. (37·78° C.) however, the beetles ceased to fly and began to seek shelter under the seaweed. It was always possible to forecast when they were about to fly as they usually first crawled to the end of a piece of seaweed. This experiment was repeated several times with similar results. All the beetles used were heavy with eggs, and several laid eggs on the seaweed.

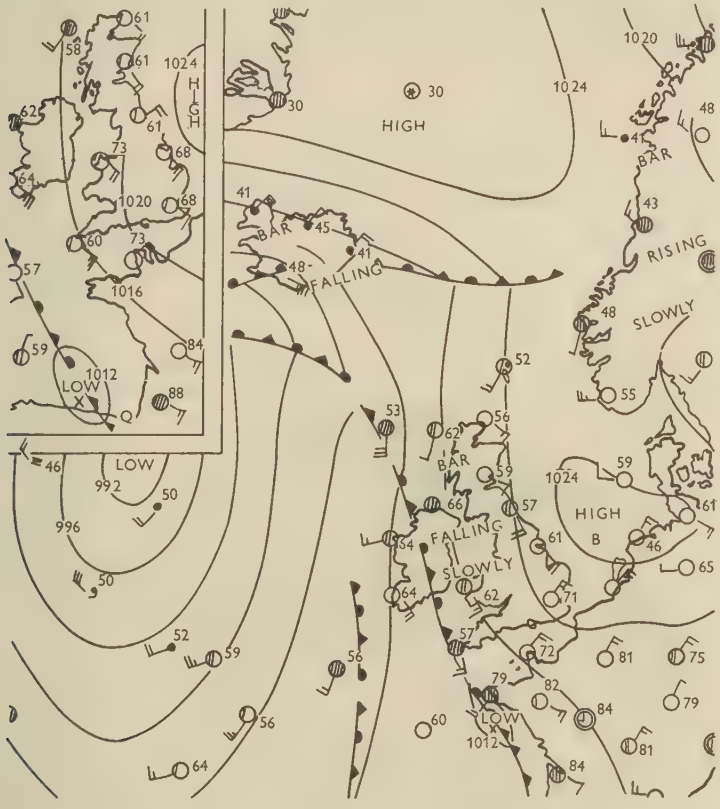

Fig. 4. Daily weather chart at 18.00 hr. 28 May 1947.

Beetles on the point of laying, or actually laying, have a bright and oily surface; whereas beetles which have ceased to lay are dull in colour and have a dried appearance. When these tests were repeated using beetles which had ceased laying, they survived only one or two days in sea water and soon became waterlogged and sank to the bottom. When placed under a bell-jar in the sun they rarely flew. As the temperature increased from 80 to 100° F. (26·67–37·78° C.) they crawled under the seaweed for shelter.

These tests suggest that beetles may drop on the sea several miles from the shore and, assisted by a favourable tide and wind, be washed up on the beaches, and under favourable temperature conditions finally fly inland. According to Feytaud (1930) the speed of a Colorado beetle in flight is approximately 8 km. (4·9 miles) per hr. It was found, in laboratory tests, that the maximum period of sustained flight was 13 min. 45 sec. From these facts the average flight range of a beetle under its own effort would appear to be about a mile. The majority of beetles found in Jersey mainly occurred within 1–1½ miles from the shore, which suggests that the normal flight range is somewhat under 2 miles.

Fig. 5. Map of Jersey showing the distribution scatter of beetles found at high-water mark between 16 and 23 May 1948.

● Heavily infested beaches. ○ Moderately infested beaches.
□ Lightly infested beaches. + Dead beetles only.

These findings, and the large numbers of dead beetles on the beaches, suggest that in May 1947, the islands of Jersey, Guernsey and Sark were invaded by beetles carried by the sea and not by direct flight from France. This was probably how islands such as Belle-Ile, off the French coast and Walcheren off Holland, became infested.

Several French fishermen reported seeing, at the end of May 1947, masses of Colorado beetles while fishing between Jersey and the Cherbourg Peninsula. On 27 May, large numbers of beetles flew over their boat, some falling on the decks and others into the sea. This occurred between the Ecrehos and Jersey, about 4 miles from the coast of Jersey (Fig. 3). Another fisherman reported large numbers of beetles floating on the sea about 5 miles off the north coast of Jersey, between the

Paternosters and the Ecrehos. At first these were thought to be floating bodies, but on closer inspection were found to be large masses of beetles piled on top of one another to a depth of 6–9 in. One of these masses was estimated to be about 8 ft. long and 6 ft. wide. None of the fishermen had seen beetles at sea before.

Of the 150 beetles found in 1947 in England, the majority were discovered after 31 May, mainly in London and the suburbs and near the Kent coast. Very few were found on potatoes. Several were found on rocks just above high-water mark along the Kent coast. These beetles with one exception were found between 31 May and 4 June, during the same period as they appeared in Jersey and Guernsey, under very similar conditions.

Factors influencing the invasion

According to Feytaud (1936) the advance of the beetle in France has been greatest towards the north and north-east and least towards the south and south-east. Prevailing winds to a large extent account for this variation in advance. The first beetle reached Normandy in 1931, and by 1933 a 'foyer' had appeared near Surtainville, far in advance of the general line of infestation. Feytaud (1939*a*) recorded that it was not until 1938 that the beetles became prevalent over the whole of the Cotentin as far as Cherbourg and the coast of Calvados. Not until 1938, therefore, did Jersey become seriously exposed to the risk of invasion from the neighbouring coast of France; and in October the following year the first outbreak was discovered.

The beetle tends to fly in swarms in the spring and autumn, when temperatures are high (Feytaud, 1936). This also occurs in America, but there it is found that the autumn flights are the more important, whereas in Europe there is evidence that the spring or early summer flights are the more dangerous. At the end of May and beginning of June 1947, the shade temperatures in France reached between 70 and 80° F. (21·11 and 26·67° C.), unusually high for that time of the year. In Jersey during the same period the shade temperatures were between 65 and 76° F. (18·33 and 24·44° C.)

On 28 May at the airport on the west of the island, the day the first beetle was reported in Jersey, the wind blew consistently from the east with an average velocity of 30 m.p.h. during most of the day, and an air temperature ranging between 59° F. (15° C.) at 06.00 hr. to 75° F. (23·89° C.) from noon to 18.00 hr. Data from Maison St Louis on the east of the island showed the wind to be blowing from the east from 18.30 hr. on 27 May until 06.00 hr. on 29 May with an average velocity of 25 m.p.h. and a maximum temperature of 79° F. (26·11° C.) on 28 May and 79·5° F. (26·39° C.) on 29 May. Temperatures are recorded in a Stephenson screen, but in the sun temperatures may be suitable for beetles taking to flight, when official records are well below 80° F. (26·67° C.).

The Meteorologist at St Louis Observatory stated that these weather conditions prevailing on 27 and 28 May, with warm air currents flowing over Jersey from the

east, were exceptional for that time of year, and only occurred about once in 10 years. This fact is important to the Channel Islands if, as seems likely, invasion from the beetle-infested Cherbourg peninsula occurs only when there is a warm air-drift in a westerly direction. Fig. 4 shows the wind drift and prevailing temperatures over Western Europe on 28 May 1947. The predominant advance of the beetle in Europe suggests that invasions of this nature are not likely to occur frequently. The meteorological evidence leaves little doubt that the beetles which invaded Jersey must have originally come from somewhere between Carteret and Coutances on the Cherbourg peninsula. Tower (1906) and Roubaud (1947) have shown that the Colorado beetle, a poor flier, flies with the wind, and into the wind for short distances, but never across it. Roubaud (1947) states that in coastal regions the influence of air currents is dangerous for the Colorado beetle and can frequently carry it out to sea.

Trouvelot (private communication) stated that conditions in the latter part of May 1947, when a period of cool weather was followed by a rapid and considerable rise in temperature, were highly favourable for flights. He suggested that a concentration, prepared in the autumn of 1946, when beetles often move about by crawling and by short flights and so collect together before hibernation, could cause a local swarm as the favourable weather occurred. In the Carteret-Barnaville district, an unusually large invasion of beetles occurred at the end of May 1947, and many were washed up on the slipway at the harbour and along the beaches. This was the first record of the occurrence of beetles on such a scale at Carteret.

The tidal flow around the Channel Islands in the Gulf of St Malo is such that if the beetles alight on the sea within 7–8 miles of the coast they may drift ashore on any of the beaches within a few days, depending on the direction of the wind. In 1948, dead beetles were washed up on the west coast of Jersey 7 days after the first live beetle had been picked up from a beach on the east of the island.

The 1948 invasion

The first indication of an invasion was the discovery of a single beetle on some seaweed on the foreshore at the extreme north-east corner of Jersey on 16 May. The following day inspectors made a search of this beach and found twenty-seven beetles, of which nine were alive. On 18 May all the beaches on the east side of the island were visited, and thousands of live beetles were found on the seaweed along the high-water mark; given suitable temperature conditions these would take to flight and again infest the island as in May 1947.

The beetles were most numerous on the beaches on the north-east coast, and, from this point, with diminishing intensity, the infestation continued along the whole of the east and part of the north coast. Counts were made at several beaches, and these varied from 13, 25, to 300 beetles/100 yd. stretch. Most of the beetles were alive and were resting on the surface of seaweed piled along the tide-mark

(Fig. 5). For several days before and after the first beetle was discovered on 16 May, the maximum day temperatures in France and Jersey were over 70° F. (21·11° C.). At the same time a strong breeze was blowing over Jersey from the north-east to east, conditions almost identical with those prevailing when Jersey was invaded in May 1947. Steps were therefore taken to spray the beaches. On 18 May the weather changed; temperatures dropped to 60° F. (15·56° C.). These adverse weather conditions temporarily prevented the beetles from flying. There was another important factor; the tides were springing so that the beetles were submerged every 12 hr., which probably accounted for the destruction of many thousands. Meanwhile, most of the beaches on the east of the island had been given two treatments with 5% D.D.T. dust along the high-tide zone. In one or two instances a third application was given. The spraying was completed by 23 May, when few live beetles could be found on the beaches. Ten days after the initial invasion, few, if any, beetles had reached the potato crops.

Discussion

Although the beetles invading the Channel Islands in 1947 and 1948 almost certainly drifted ashore from the sea, the possibility of future infestations by flying beetles cannot be excluded, though it is unlikely. Feytaud (1939*b*) records beetles having flown 120–150 km. Such an invasion, however, would necessitate the beetles starting from a point on the French coast, so situated that an air stream would carry them across one or other of the islands. Since prolonged flights of such a nature are determined largely by air currents, the possibilities of the beetles alighting exactly 14 or 30 miles from their point of origin are very slight indeed. Because the beetles can invade small land surfaces indirectly from the sea, the potential zone of invasion is very much increased.

May and June appear to be the most critical periods, just after the hibernants have emerged and start flying, seeking food plants in preparation for laying eggs. Laboratory tests indicate that these beetles can survive more successfully in sea water than beetles which have ceased to lay, largely due to the protective layer of oil which covers the epidermis of the exoskeleton of beetles not long emerged from hibernation. They also show more propensity to fly when the temperatures are raised to 80–85° F. (26·67–29·44° C.) than beetles which have passed their egg-laying peak. Spring migrants are therefore the more likely to cause an indirect invasion.

The 1947 and 1948 invasions of Jersey indicate that if particular attention were paid to the meteorological conditions prevailing in France during May and June Colorado-beetle flights could probably be forecast. When shade temperatures rise above 70° F. (21·11° C.), beaches facing the Continent should be searched so that beetles are noticed on arrival and can be killed before they move inland. Experience gained in Jersey showed that hand-dusting machines were too slow and many

beaches too inaccessible for sprayers to be used satisfactorily. To check a threatened invasion successfully, all beaches should be sprayed immediately, before the beetles start to fly, and it seems that this can only be done successfully from the air.

The writer wishes to express his thanks to Dr T. Small for his interest and advice; to Mr E. Gruchy for his assistance in the field. Thanks are also due to the Director of the Meteorological Office, Air Ministry, and the Controller, H.M. Stationery Office, London, for permission to reproduce part of the *Daily Weather Report* for 28 May 1947.

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RELATION BETWEEN PARTICLE SIZE AND SHAPE OF INSECTICIDAL SUSPENSIONS AND THEIR CONTACT TOXICITY

II. D.D.T. AND ROTENONE SUSPENSIONS AGAINST *ORYZAEPHILUS SURINAMENSIS* L., WITH SOME TIME-MORTALITY STUDIES

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(With Plate 13 and 4 Text-figures)

Methods already worked out for the preparation and testing of aqueous D.D.T. suspensions against *Tribolium castaneum*, by a dipping method, have been applied to *Oryzaephilus surinamensis*, giving similar results, i.e. toxicity increases with increase in particle size.

In the same way, the precipitation of rotenone by exchange of solvents leads to the formation of simple aqueous suspensions. The theory of precipitation is described and methods are given of preparing five types of suspension: colloidal rotenone, a suspension of small elongated plates, a suspension containing small hexagonal plates in aggregates, and two suspensions containing hexagonal plate-shaped crystals of different sizes.

These were tested, by dipping, against *Oryzaephilus surinamensis* L. Within the range of crystal sizes up to about 150 μ, toxicity is inversely related to the size of particle in suspension. The variation in median lethal concentration obtained in this way is of the order of 600 times. Crystal shape seems to be unimportant. Similar results were obtained with fine suspensions, using a spraying method.

The variation of mortality with time was also studied, using D.D.T. against *Tribolium castaneum* and rotenone against *Oryzaephilus surinamensis*. In the former case, both colloidal and crystalline D.D.T. show progressively increasing kills with the passage of time. Crystalline rotenone behaves similarly, but colloidal rotenone gives an initial paralytic effect, followed by recovery of the insects.

1. Introduction

In Part I (McIntosh, 1947*b*), methods of preparing and testing various simple aqueous suspensions of D.D.T. were described. It was shown that the toxicity of D.D.T. suspensions increased with increasing size of crystal in suspension, and that these differences in toxicity were paralleled by retention of greater amounts of poison from coarser than from finer suspensions. Two types of suspension, containing extreme sizes of particle, were of almost identical toxicity when comparisons were made on the basis of weight of D.D.T. retained by the insects and not on the basis of percentage concentration in suspension. Frequent reference will be made to this earlier paper (McIntosh, 1947*b*) as 'Part I'.

One of the objects of the present series being a comparison of the effects of

particle size on toxicity of different poisons, rotenone was selected as a second poison for study. The test-subject used for D.D.T. in Part I (*Tribolium castaneum*) is exceptionally resistant to rotenone. As it is desirable to make a comparison of the two poisons on the same test-subject, a few experiments were made with D.D.T. against adult *Oryzaephilus surinamensis* L., a species which is susceptible to rotenone. These experiments were carried out using some of the suspensions described in Part I.

Pure rotenone and derris extracts have been used as suspensions for a considerable time, and as long ago as 1923 it was suspected by Fryer, Stenton, Tattersfield & Roach that toxicity varied inversely with particle size. Later workers (Cahn, Phipers & Boam, 1938; Richardson, 1935; Tattersfield & Martin, 1938) have strengthened this view, although in no case were critical measurements made of either toxicity or particle size.

As pure rotenone is readily obtained, the methods employed with D.D.T. can, with suitable modification, be applied to the preparation and testing of somewhat similar uniform suspensions of rotenone.

2. D.D.T. suspensions against *Oryzaephilus surinamensis*

Toxicity tests

Oryzaephilus surinamensis L. were reared on rolled oats at 32° C., and adults were used in the standard dipping method (Part I) with a few minor modifications. Batches of fifteen insects were dipped for $1\frac{1}{4}$ min. at 27° C., using 8 ml. of suspension to obtain three replicates at each concentration. Owing to the smallness of the insects, it is necessary to use two layers of muslin to contain them in the tubes after dipping. At the end of the dipping period, one of the stoppers is replaced by a single layer of muslin, and the suspension is drained through this; the other stopper is replaced by double muslin, and the single layer at the opposite end is then covered with a second muslin.

The insects were kept for 24 hr. at 27° C., without food, before inspection.

Results of toxicity tests

Tests have been made with colloidal D.D.T. (type I of Part I) and the three needle suspensions (types IV, V and VI of Part I). As before, the probit method has been used to assess the results.

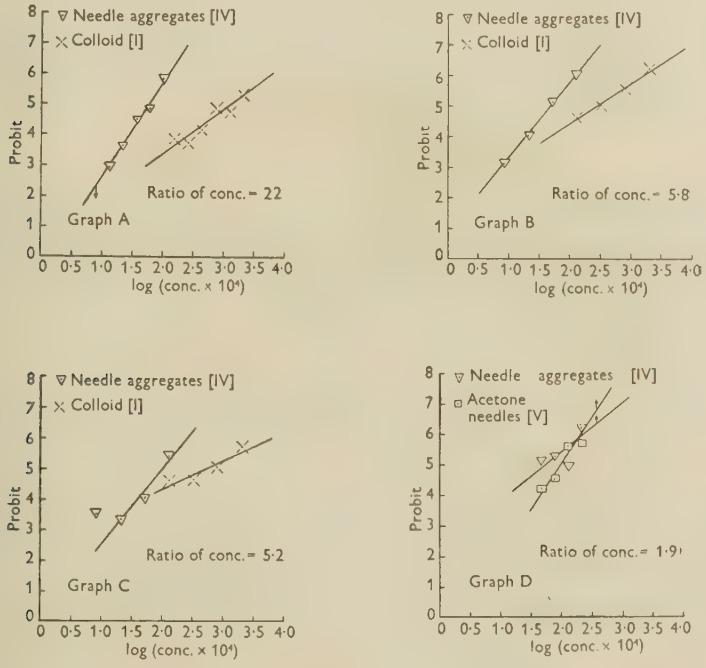
The results of the tests are given in Text-figs. 1 and 2, graphs A–E. Each of the lines is the calculated 'best fit' for the group of points in question. The symbols ↑ and ↓ signify concentrations at which were obtained kills of 100 and 0% respectively. Some details of the probit analyses and size measurements are given in Table 1. The marks ' × ' show the suspension types compared in the various tests. The equations take the form $y = bx + c$, where y = probit kill, b = gradient of line, x = log (percentage concentration × 10^4) and c = constant. The values of x when

$y = 5$ [i.e. log (median lethal concentration $\times 10^4$) or log (M.L.C.)] are calculated from the equations. The figure for 'mean ratio of M.L.C.' is the geometrical mean of the individual ratios of median lethal concentrations. All the ratios are given with the concentration of the less toxic preparation as numerator.

TABLE 1. *Results of tests A–E. Details of probit lines showing effect of particle size of D.D.T. suspensions on their toxicity to adult* Oryzaephilus surinamensis

	Suspension type						Particulars of probit lines						
Test	Colloid I	Needle agg. IV	Short acetone needles V	Short alcohol needles VI	Types tested	Mean size in μ	Equation	Standard error in b	Log [M.L.C. $\times 10^4$]	Difference in logs	Mean difference in logs	(Mean) ratio of [M.L.C.]	Test
A	×	—	—	—	I	—	$y = 1{\cdot}45x + 0{\cdot}50$	$1{\cdot}45 \pm 0{\cdot}26$	$3{\cdot}11 \pm 0{\cdot}08$	$1{\cdot}35 \pm 0{\cdot}08$			A
	—	×	—	—	IV	500	$y = 3{\cdot}11x - 0{\cdot}48$	$3{\cdot}11 \pm 0{\cdot}36$	$1{\cdot}76 \pm 0{\cdot}04$				
B	×	—	—	—	I	—	$y = 1{\cdot}31x + 1{\cdot}81$	$1{\cdot}31 \pm 0{\cdot}25$	$2{\cdot}44 \pm 0{\cdot}09$	$0{\cdot}76 \pm 0{\cdot}10$	$0{\cdot}94$	8·7	B
	—	×	—	—	IV	407	$y = 2{\cdot}47x + 0{\cdot}86$	$2{\cdot}47 \pm 0{\cdot}36$	$1{\cdot}68 \pm 0{\cdot}05$				
C	×	—	—	—	I	—	$y = 0{\cdot}93x + 2{\cdot}47$	$0{\cdot}93 \pm 0{\cdot}26$	$2{\cdot}71 \pm 0{\cdot}12$	$0{\cdot}71 \pm 0{\cdot}14$			C
	—	×	—	—	IV	416	$y = 2{\cdot}44x + 0{\cdot}12$	$2{\cdot}44 \pm 0{\cdot}48$	$2{\cdot}00 \pm 0{\cdot}07$				
D	—	×	—	—	IV	426	$y = 1{\cdot}58x + 2{\cdot}30$	$1{\cdot}58 \pm 0{\cdot}40$	$1{\cdot}71 \pm 0{\cdot}11$	$0{\cdot}27 \pm 0{\cdot}12$		1·9	D
	—	—	×	—	V	128	$y = 3{\cdot}07x - 1{\cdot}06$	$3{\cdot}07 \pm 0{\cdot}38$	$1{\cdot}98 \pm 0{\cdot}04$				
E	—	×	—	—	IV	349	$y = 1{\cdot}92x + 1{\cdot}48$	$1{\cdot}92 \pm 0{\cdot}23$	$1{\cdot}83 \pm 0{\cdot}05$	$0{\cdot}43 \pm 0{\cdot}06$		2·7	E
	—	—	—	×	VI	46	$y = 2{\cdot}92x - 1{\cdot}59$	$2{\cdot}92 \pm 0{\cdot}30$	$2{\cdot}26 \pm 0{\cdot}03$				

All toxicity ratios are given with the concentration of the less toxic preparation as numerator.



Text-fig. 1. Graphs A–D. Mortality of adult *O. surinamensis* produced by D.D.T. suspensions in dipping tests.

Text-fig. 1, graphs A, B and C, shows results obtained from tests of needle aggregates (mean size 440 μ) against colloidal suspension. Graph D shows a comparison of needle aggregates and short acetone needles (128 μ), and text-fig. 2, graph E, a comparison of needle aggregates and short alcohol needles (46 μ). With *O. surinamensis* it is common to obtain a small kill (*c.* 4%) in the controls, and corrections have been made for this where necessary.

In this series, the effects of crystal shape, which were minor in the case of *Tribolium castaneum*, have not been examined. The ratios obtained, although perhaps smaller, give the same general picture for the effect of crystal size on toxicity, i.e. toxicity increases with crystal length. There is no indication of an optimum size.

3. Rotenone suspensions against *Oryzaephilus surinamensis*

Preparation of suspensions

Precipitation of rotenone

Although the general principles described in Part I apply to the preparation of suspensions of any solid material by condensation processes, the methods actually employed must be suited to the properties of the substance being precipitated. Rotenone has the tendency to crystallize from supersaturated solution far more rapidly than D.D.T. under similar circumstances; in addition, the size and shape of crystals are more difficult to control.

Materials

The materials used were: purified crystalline rotenone (m.p. 163–4° C.), distilled water, 95% alcohol, rectified acetone, sulphonated lorol (Ronsheim & Moore) and saponin (B.D.H.). With the exception of type I, the preparations described below are for suspensions containing 0·1% rotenone.

Apparatus and size estimation

All preparations are carried out at room temperature.

No special equipment is needed for type I. The apparatus described in Part I, p. 593, is suitable for preparation of type II rotenone suspensions.

For types III, IV and V, the screw clip is unnecessary, and the capillary tube is replaced by a plain glass tube with an orifice of diameter 2 mm. A 500 ml. bolt-head flask is to be preferred as a great amount of foam is produced.

The mean crystal size is estimated by measuring twenty crystals on a microscope slide. With type V, the maximum length and breadth are taken.

Addition of rotenone solution to aqueous detergent solution

Type I (*colloidal rotenone*)

Colloidal rotenone is at once formed if rotenone in water-miscible solution is added to water or an aqueous solution of a detergent. The conditions favouring a high degree of dispersion can, as in the case of D.D.T., be predicted from von Weimarn's equation (Part I). In the presence of detergents, or in the complete absence of any surface-active material, crystal growth takes place very rapidly. Using 0·1% sulphonated lorol and an alcoholic rotenone solution to give a final concentration of 0·01%, a clear solution is formed at first. This rapidly becomes viscous, and then gradually turbid and thinner again as visible flocculation begins. Growth is complete within $1\frac{1}{2}$ hr.

The same sequence occurs at much the same rate with many other synthetic detergents. The growth rate can be decreased by the presence of natural protective colloids, such as gum arabic, gum tragacanth, pectin or saponin. Saponin is the most efficient.

The highest concentration of rotenone required is commonly in the region of 0·001%. 0·1 g. saponin is dissolved in 95 ml. water. A solution is prepared containing 0·02 g. rotenone in 100 ml. alcohol, and 5 ml. of this are pipetted into the saponin solution, which is simultaneously stirred by hand. This gives a clear colloidal suspension, containing 0·001% rotenone, 0·1% saponin and 5% alcohol.

Addition of detergent solution to rotenone solution

Type II (*large hexagons*)

The method is identical with that described for D.D.T. needle aggregates (Part I) with the differences that rotenone is substituted for D.D.T. and the speed of addition is 60 drops/min. Precipitation begins in about 12 min., and after 20 min. the rate of addition can be increased. The suspension contains rotenone 0·1%, sulphonated lorol 0·1% and acetone 10%. Pl. 13, fig. 1, shows typical hexagonal plate-shaped crystals, of mean size about $150 \times 115 \mu$.

Type III (*small elongated plates*)

By increasing the rates of mixing and stirring and by using alcohol-acetone mixtures to decrease the solubility of rotenone in the system, suspensions containing smaller crystals can be produced. However, only a small reduction in size is to be obtained in this way, and great variation in size and shape of crystal occurs from one preparation to another. Further, extreme rates of mixing give rise not to the direct precipitation of rotenone, but to the formation of supersaturated solutions, from which growth occurs fairly rapidly. It is the crystallization of these highly viscous systems that is so difficult to control. The size of crystal obtained is very

sensitive to the rate of stirring during the viscous stage, in which nucleation takes place; the only way to obtain reproducible results is to stir the mixture violently until growth has reached an advanced stage.

In the bolt-head flask 0·2 g. rotenone is dissolved in 20 ml. of a solvent mixture (alcohol:acetone = 4:1). 180 ml. water containing 0·2 g. sulphonated lorol are placed in the funnel. With the maximum stirring available (*c.* 2000 r.p.m.) the entire detergent solution is now run into the solvent mixture through the wide glass jet, with the tap fully open. A pronounced milkiness appears after about 30 sec. The vigorous stirring is continued for 45 min. and the suspension is set aside overnight. The composition is rotenone 0·1%, sulphonated lorol 0·1%, alcohol 8% and acetone 2%. The small, rather elongated plate-shaped crystals are about $20 \times 6\mu$ (Pl. 13, fig. 2).

Type IV (*small hexagons*)

Harbury (1947) has pointed out that when salts crystallize from supersaturated solution, the induction period, or time required before a new phase separates, can be expressed by an equation of the form

$$I = \frac{K}{C^2},$$

where I = induction period, K = constant, and $C = Q/L$, Q being the concentration of precipitating substance available in solution, and L being the solubility of large crystals of precipitate. Something of the same nature may be expected to hold in the present case. In the third method, stirring must be continued for some 45 min. The period of stirring can be curtailed if the amount of sulphonated lorol solution added to the rotenone solution is reduced.

Thus, a solution of rotenone in solvent mixture is contained in the flask as before. 50 ml. of a solution of 0·2 g. sulphonated lorol in 180 ml. water are placed in the funnel, and added to the rotenone solution with maximum stirring, which is continued for 10 min. before the remaining 130 ml. of the detergent solution are added in the same way. The mixture is allowed to stand overnight before use; it contains rotenone 0·1%, sulphonated lorol 0·1%, alcohol 8% and acetone 2%. Typical small hexagonal plate-like crystals are shown in Pl. 13, fig. 3. The mean size is about $25 \times 15\mu$.

Test V (*hexagon aggregates*)

In both the above methods, growth, though advanced, is not complete by the end of the stirring period; the suspensions must be left to ripen overnight at room temperature. If the mixing is incomplete, as in the fourth method, and the remaining sulphonated lorol solution withheld for some time from the crystallizing mixture, a different type of suspension is formed.

The previous preparation is followed until the end of the 10 min. of vigorous

stirring. The mixture and the 130 ml. detergent solution are kept separately overnight, and mixed in the same way, but with moderate stirring, after 15–18 hr. The composition is again rotenone 0·1%, sulphonated lorol 0·1%, alcohol 8%, and acetone 2%. The individual hexagons have fused together into plate-shaped clusters of about $60 \times 45 \mu$ (Pl. 13, fig. 4).

Transference to other media

It is desirable to carry out the biological tests of crystalline suspensions in a medium corresponding to the composition of the colloidal suspension (0·1% saponin, 5% alcohol); Martin (1940) has shown that the toxicity of colloidal derris suspensions to *O. surinamensis* can be decreased by a factor of four if the medium is changed from 0·5% sulphonated lorol to 0·5% saponin, both containing 10% alcohol.

The rotenone crystals in the suspensions of types II–V all settle out fairly rapidly. It is convenient to allow the ripening to proceed in stoppered measuring cylinders. When sedimentation is complete, the liquid can be drawn off with a filter pump and replaced by a suitable medium. The crystals are easily redispersed by shaking.

Toxicity tests

Methods

Again, the dipping method (McIntosh, 1947*a*), and occasionally the Potter tower (1941), have been used, in conjunction with probit analysis.

Dipping procedure

The test subjects were adult *Oryzaephilus surinamensis* L., reared at 32° C. on rolled oats. Batches of fifteen insects were dipped for $1\frac{1}{4}$ min. at 27° C., and 8 ml. of suspension were used to obtain three replicates at each concentration. With type II (large hexagons), the suspension was renewed for each dipping tube. Double muslin was used, as before. Until inspection, the insects were kept 24 hr. at 32° C.

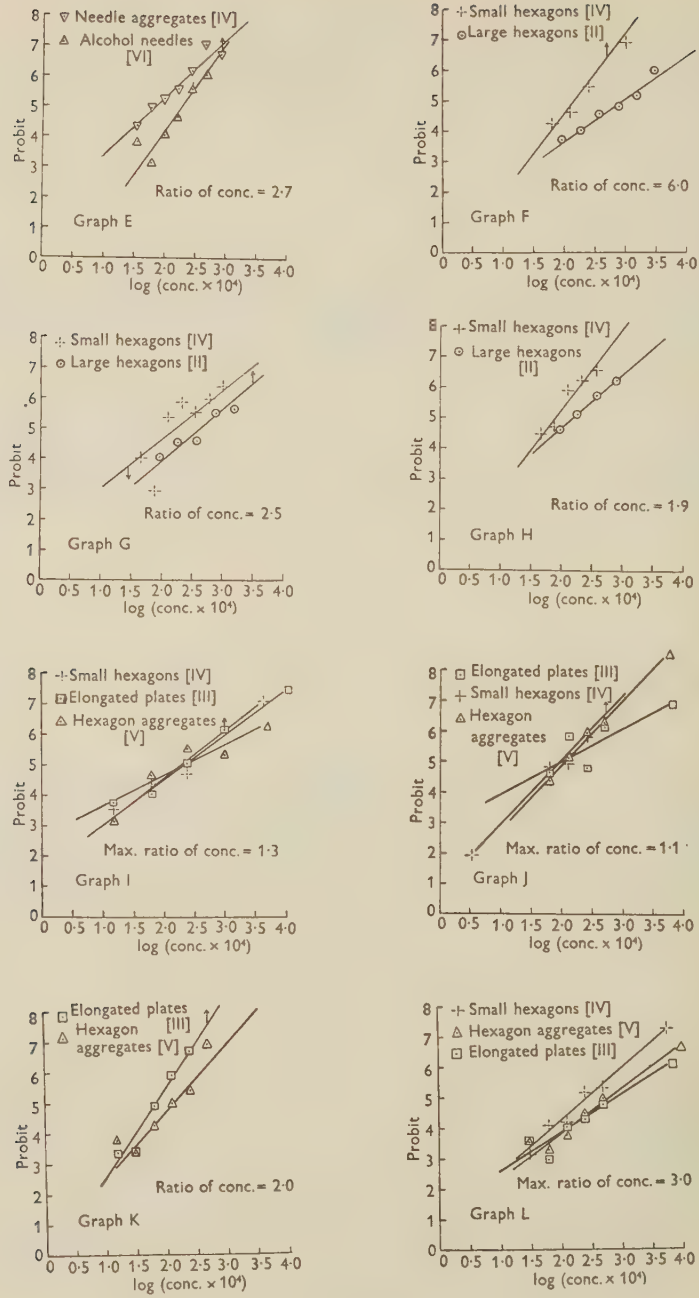
Results of toxicity tests

Results using the dipping method

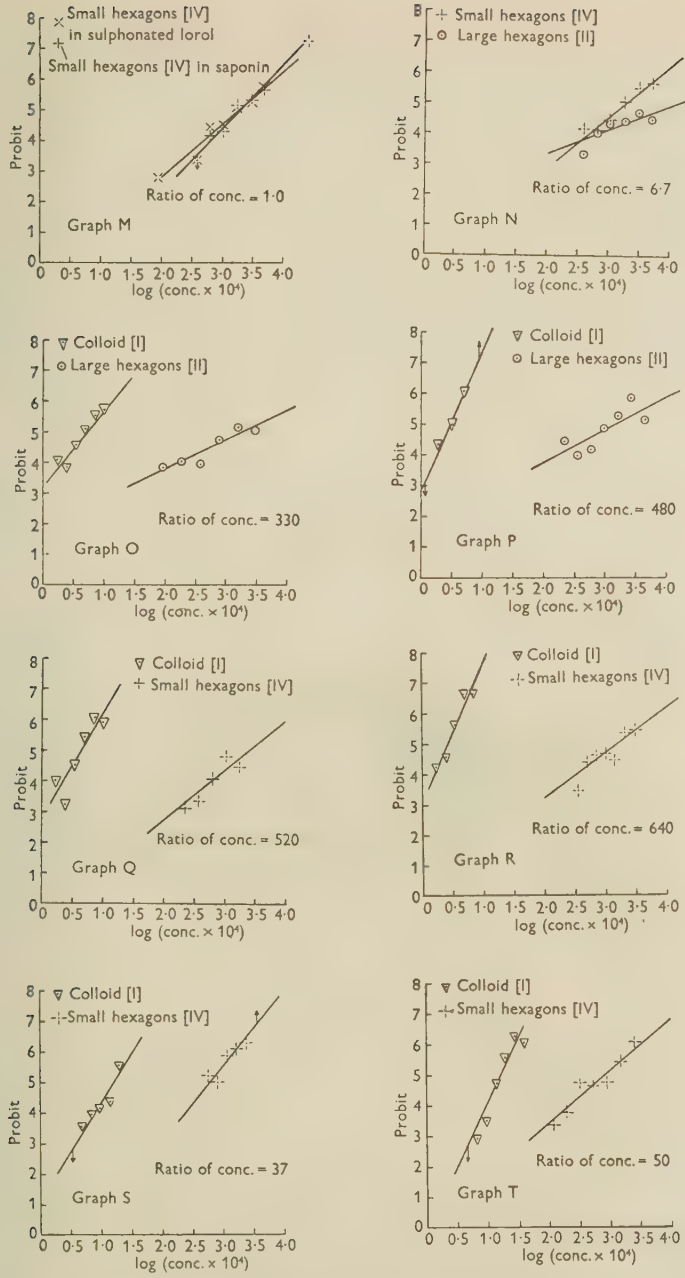
These results are given in Text-figs. 2 and 3, graphs F–R, and details of the probit lines in Table 2. The ratios obtained, although consistent qualitatively, show bigger numerical variations than the results of Part I.

The suspensions were tested in order of decreasing particle size, and the earlier tests (F–L) were made in a medium comprising 0·1% sulphonated lorol, 10% solvent. In tests N–R the medium was 0·1% saponin, 5% alcohol.

Tests F, G and H show that large hexagons (mean size $= 157 \times 118 \mu$) are less toxic than small hexagons (mean size $= 21 \times 13 \mu$). From tests I, J, K and L it is seen that types III, IV and V do not differ in toxicity.



Text-fig. 2. Graph E. Mortality of adult *O. surinamensis* produced by D.D.T. suspensions in dipping tests. Graphs F–L. Mortality of adult *O. surinamensis* produced by suspensions of rotenone in dipping tests.



Text-fig. 3. Graphs M–R. Mortality of adult *O. surinamensis* produced by suspensions of rotenone in dipping tests. Graphs S and T. Mortality of adult *O. surinamensis* produced by suspensions of rotenone in spraying tests.

TABLE 2. *Results of dipping tests F–R and of spraying tests S and T. Details of probit lines showing effect of particle size of rotenone suspensions on their toxicity to adult* Oryzaephilus surinamensis.

Test	Suspension type: Colloid I	Large hex. II	Small elong. plates III	Small hex. IV	Hex. agg. V	Types tested	Mean size in μ	Particulars of probit lines: Equation	Standard error in b	Log [M.L.C. × 10^4]	(Max.) Difference in logs	Mean difference in logs	(Mean) ratio of [M.L.C.]	Test
F	—	×	—	—	—	II	153 × 110	$y = 1{\cdot}41x + 0{\cdot}84$	$1{\cdot}41 \pm 0{\cdot}19$	$2{\cdot}95 \pm 0{\cdot}07$	$0{\cdot}78 \pm 0{\cdot}08$			
	—	—	—	×	—	IV	26 × 17	$y = 2{\cdot}62x - 0{\cdot}69$	$2{\cdot}62 \pm 0{\cdot}31$	$2{\cdot}17 \pm 0{\cdot}04$				F
G	—	×	—	—	—	II	187 × 153	$y = 1{\cdot}70x + 0{\cdot}55$	$1{\cdot}70 \pm 0{\cdot}21$	$2{\cdot}62 \pm 0{\cdot}06$	$0{\cdot}40 \pm 0{\cdot}09$	0·49	3·1	
	—	—	—	×	—	IV	20 × 10	$y = 1{\cdot}61x + 1{\cdot}42$	$1{\cdot}61 \pm 0{\cdot}32$	$2{\cdot}22 \pm 0{\cdot}07$				G
H	—	×	—	—	—	II	130 × 91	$y = 1{\cdot}79x + 1{\cdot}11$	$1{\cdot}79 \pm 0{\cdot}37$	$2{\cdot}17 \pm 0{\cdot}08$				
	—	—	—	×	—	IV	15 × 9	$y = 2{\cdot}68x - 0{\cdot}06$	$2{\cdot}68 \pm 0{\cdot}44$	$1{\cdot}89 \pm 0{\cdot}05$	$0{\cdot}28 \pm 0{\cdot}10$			H
I	—	—	×	—	—	III	29 × 6	$y = 1{\cdot}47x + 1{\cdot}59$	$1{\cdot}47 \pm 0{\cdot}21$	$2{\cdot}32 \pm 0{\cdot}08$				
	—	—	—	×	—	IV	15 × 9	$y = 1{\cdot}54x + 1{\cdot}50$	$1{\cdot}54 \pm 0{\cdot}20$	$2{\cdot}27 \pm 0{\cdot}08$	$0{\cdot}11 \pm 0{\cdot}15$		1·3	
	—	—	—	—	×	V	64 × 44	$y = 0{\cdot}98x + 2{\cdot}67$	$0{\cdot}98 \pm 0{\cdot}20$	$2{\cdot}38 \pm 0{\cdot}13$				I
J	—	—	×	—	—	III	21 × 5	$y = 1{\cdot}08x + 2{\cdot}86$	$1{\cdot}08 \pm 0{\cdot}34$	$1{\cdot}98 \pm 0{\cdot}13$				
	—	—	—	×	—	IV	29 × 22	$y = 2{\cdot}08x + 0{\cdot}87$	$2{\cdot}08 \pm 0{\cdot}35$	$1{\cdot}99 \pm 0{\cdot}06$	$0{\cdot}06 \pm 0{\cdot}14$		1·1	
	—	—	—	—	×	V	73 × 50	$y = 2{\cdot}14x + 0{\cdot}63$	$2{\cdot}14 \pm 0{\cdot}38$	$2{\cdot}04 \pm 0{\cdot}06$				J
K	—	—	×	—	—	III	24 × 5	$y = 2{\cdot}91x - 0{\cdot}32$	$2{\cdot}91 \pm 0{\cdot}28$	$1{\cdot}83 \pm 0{\cdot}04$				
	—	—	—	—	×	V	63 × 38	$y = 2{\cdot}16x + 0{\cdot}40$	$2{\cdot}16 \pm 0{\cdot}28$	$2{\cdot}13 \pm 0{\cdot}05$	$0{\cdot}30 \pm 0{\cdot}07$		2·0	
L	—	—	×	—	—	III	30 × 8	$y = 1{\cdot}26x + 1{\cdot}31$	$1{\cdot}26 \pm 0{\cdot}27$	$2{\cdot}93 \pm 0{\cdot}17$				
	—	—	—	×	—	IV	21 × 13	$y = 1{\cdot}69x + 0{\cdot}85$	$1{\cdot}69 \pm 0{\cdot}28$	$2{\cdot}46 \pm 0{\cdot}07$	$0{\cdot}47 \pm 0{\cdot}18$		3·0	
	—	—	—	—	×	V	40 × 25	$y = 1{\cdot}45x + 0{\cdot}94$	$1{\cdot}45 \pm 0{\cdot}26$	$2{\cdot}80 \pm 0{\cdot}12$				L
M		S. lorol		×	—	IV	20 × 12	$y = 1{\cdot}71x - 0{\cdot}62$	$1{\cdot}71 \pm 0{\cdot}26$	$3{\cdot}29 \pm 0{\cdot}05$				
		Saponin		×	—	IV	25 × 13	$y = 2{\cdot}07x - 1{\cdot}84$	$2{\cdot}07 \pm 0{\cdot}27$	$3{\cdot}31 \pm 0{\cdot}05$	$0{\cdot}02 \pm 0{\cdot}07$		1·0	M
N	—	×	—	—	—	II	107 × 77	$y = 0{\cdot}80x + 1{\cdot}74$	$0{\cdot}80 \pm 0{\cdot}28$	$4{\cdot}09 \pm 0{\cdot}32$				
	—	—	—	×	—	IV	19 × 12	$y = 1{\cdot}67x - 0{\cdot}45$	$1{\cdot}67 \pm 0{\cdot}27$	$3{\cdot}26 \pm 0{\cdot}06$	$0{\cdot}83 \pm 0{\cdot}32$		6·7	N
O	×	—	—	—	—	I	—	$y = 2{\cdot}43x + 3{\cdot}25$	$2{\cdot}43 \pm 0{\cdot}48$	$0{\cdot}72 \pm 0{\cdot}05$				
	—	×	—	—	—	II	103 × 80	$y = 0{\cdot}95x - 1{\cdot}92$	$0{\cdot}95 \pm 0{\cdot}20$	$3{\cdot}24 \pm 0{\cdot}13$	$2{\cdot}52 \pm 0{\cdot}14$			O
P	×	—	—	—	—	I	—	$y = 4{\cdot}63x + 2{\cdot}78$	$4{\cdot}63 \pm 0{\cdot}62$	$0{\cdot}48 \pm 0{\cdot}03$		2·60	400	
	—	×	—	—	—	II	179 × 135	$y = 1{\cdot}06x + 1{\cdot}65$	$1{\cdot}06 \pm 0{\cdot}22$	$3{\cdot}16 \pm 0{\cdot}09$	$2{\cdot}68 \pm 0{\cdot}09$			P
Q	×	—	—	—	—	I	—	$y = 3{\cdot}46x + 2{\cdot}76$	$3{\cdot}46 \pm 0{\cdot}47$	$0{\cdot}65 \pm 0{\cdot}03$				
	—	—	—	×	—	IV	21 × 14	$y = 1{\cdot}63x - 0{\cdot}48$	$1{\cdot}63 \pm 0{\cdot}50$	$3{\cdot}36 \pm 0{\cdot}14$	$2{\cdot}71 \pm 0{\cdot}10$			Q
R	×	—	—	—	—	I	—	$y = 4{\cdot}58x + 3{\cdot}07$	$4{\cdot}58 \pm 0{\cdot}62$	$0{\cdot}42 \pm 0{\cdot}03$		2·76	580	
	—	—	—	×	—	IV	29 × 20	$y = 1{\cdot}47x + 0{\cdot}27$	$1{\cdot}47 \pm 0{\cdot}33$	$3{\cdot}23 \pm 0{\cdot}07$	$2{\cdot}81 \pm 0{\cdot}07$			R
S	×	—	—	—	—	I	—	$y = 3{\cdot}15x + 1{\cdot}17$	$3{\cdot}15 \pm 0{\cdot}48$	$1{\cdot}22 \pm 0{\cdot}04$				
	—	—	—	×	—	IV	22 × 14	$y = 2{\cdot}47x - 1{\cdot}87$	$2{\cdot}47 \pm 0{\cdot}38$	$2{\cdot}78 \pm 0{\cdot}06$	$1{\cdot}56 \pm 0{\cdot}07$			S
T	×	—	—	—	—	I	—	$y = 4{\cdot}17x - 0{\cdot}08$	$4{\cdot}17 \pm 0{\cdot}34$	$1{\cdot}22 \pm 0{\cdot}02$		1·63	43	
	—	—	—	×	—	IV	18 × 9	$y = 1{\cdot}70x + 0{\cdot}05$	$1{\cdot}70 \pm 0{\cdot}24$	$2{\cdot}92 \pm 0{\cdot}05$	$1{\cdot}70 \pm 0{\cdot}06$			T

All toxicity ratios are given with the concentration of the less toxic preparation as numerator.

In tests M and N, the effect of medium on toxicity has been studied. Small hexagons have been tested in two media analogous to those used by Martin (see p. 541), i.e. (in this case) 0·1 % sulphonated lorol and 0·1 % saponin, both containing 5 % alcohol. As the toxicity ratio obtained in test M was 1·0, it seems that the effect noted by Martin (1940), for derris suspensions against *O. surinamensis*, does not apply to crystalline suspensions of rotenone against the same test-subjects. Further, graph N shows large hexagons tested against small in saponin medium, giving a toxicity ratio not much different from those obtained in sulphonated lorol medium, especially in test F. Thus it has been assumed that the small differences between the media do not influence either the relative or absolute values of toxicity. There is always the chance that the toxicity of colloidal rotenone may depend on the medium, but for reasons mentioned earlier (p. 539), it is unfortunately not possible to prove or disprove this with pure rotenone. (Stable derris resin suspensions can be prepared using sulphonated lorol.)

The results of tests O, P, Q and R are rather confusing, but this may be due to the fact that Q and R were carried out at a later date than O and P, using younger cultures of insects. The tests show that colloidal rotenone is very much more potent than large hexagons; and that the ratio obtained with colloid against small hexagons is greater still, even though small hexagons are known to be more toxic than large.

Results using the Potter tower

Tests made with this apparatus differed from the above only in the method of application of the insecticide. The environmental conditions of the insects before and after poisoning were the same in both cases.

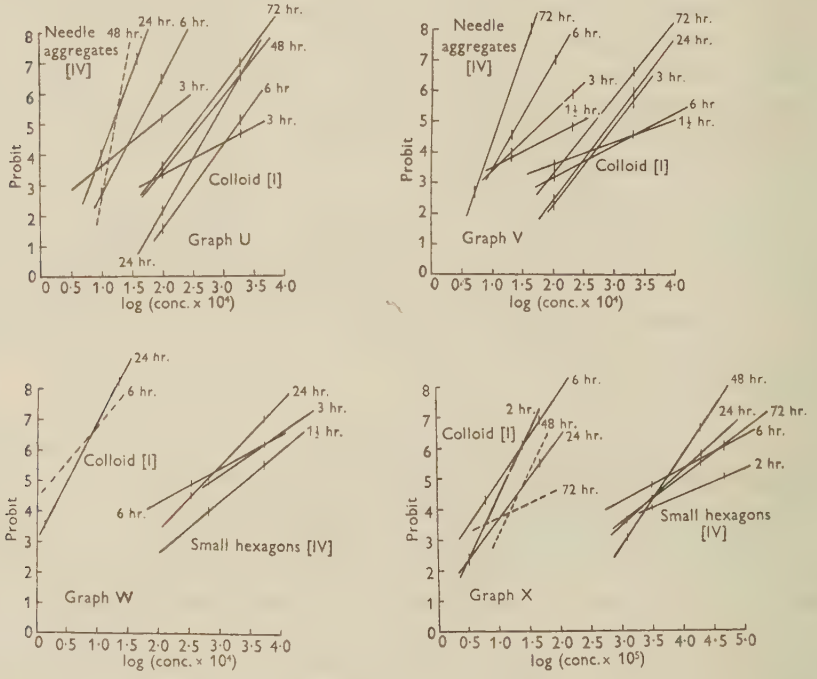
The suspensions of smaller crystals pass through the nozzle of the tower without damage. Comparisons of colloidal rotenone and small hexagons, both in 0·1 % saponin and 5 % alcohol, are seen in Text-fig. 3, graphs S and T, and in Table 2. The toxicity ratios are considerably smaller than in tests Q and R. It will be recalled that, in Part I, the Potter tower gave rather smaller differences than the dipping method.

4. Time-mortality tests

As both D.D.T. and rotenone are slow-acting poisons, not credited with any knock-down effect, and as toxicity comparisons have hitherto been made at arbitrary intervals of time after treatment, it is of some interest to determine how the kills vary with time.

For both poisons, a slightly modified dipping procedure was adopted. Groups of fifty insects were dipped for 1¼ min. at 27° C., using 8 ml. suspension. One group was used for each concentration of poison. The insects were kept at appropriate temperatures until inspection at various lengths of time after dipping. For each probit line a separate set of insects was employed, and was not kept after being inspected once.

With D.D.T., colloid (I) and needle aggregates (IV) were used against *Tribolium castaneum*, kept at 27° C. after treatment; with rotenone, colloid (I) and small hexagons (IV) were used against *Oryzaephilus surinamensis*, kept at 32° C. after treatment. Text-fig. 4, graphs U and V (D.D.T.) and graphs W and X (rotenone), show the probit lines obtained. Each line represents four points, but to avoid confusion none of the points are shown. The vertical marks crossing each line,



Text-fig. 4. Graphs U and V. Mortality of adult *T. castaneum* produced by D.D.T. needle aggregates and colloid at various lengths of time after dipping. Graphs W and X. Mortality of adult *O. surinamensis* produced by rotenone small hexagons and colloid at various lengths of time after dipping.

however, show the highest and lowest concentrations employed. The broken lines are fixed by only two points each. The time (hr.) between dipping and inspection is shown at the end of each line.

It is seen that rotenone small hexagons, as well as both types of D.D.T. suspension, give kills which gradually increase with time. In most cases there are concomitant increases in slope. With colloidal D.D.T. there is some recovery from treatment with the lower concentrations.

Colloidal rotenone, however, shows the opposite effect from the three other types,

viz. a marked recovery of the insects at all concentrations, and there is even some evidence that the lines become flatter.

5. Discussion

It has been mentioned that the toxicity differences, obtained in Part I, showed that the increasing toxicity with increasing size of crystal in suspension was paralleled by more efficient retention of D.D.T., on the bodies of *Tribolium castaneum*, from coarser than from finer suspensions. With *Oryzaephilus surinamensis*, a suspension of large D.D.T. crystals is in the same way more toxic than a fine suspension, and it is to be supposed that retention efficiency is again a contributory cause, although direct tests were not made to confirm this. It also seems reasonable to suppose that *O. surinamensis* would have given time-mortality results similar to those given by *Tribolium castaneum*.

The results obtained in these experiments with D.D.T. seem to be in general agreement with those reported by other authors in experiments in which the size of crystal of D.D.T. was the only variable. Thus, it is known that the method of application can affect the crystal form of D.D.T. in films (Morrison, 1945; Starr, 1945), and that this can influence the toxicity, the larger crystals being more effective (Ebeling, 1945; Vickers, 1946). Nasir (1947) has, however, stated that the most effective size of crystal (against pests of stored products) is 5 μ; and Van den Hende (1948) was unable to find any difference in potency between crystals of 10 μ and needles 1–2 mm. long, tested as films against *Calandra oryzae*, *Tribolium confusum* and *Macrosiphoniella chrysanthemi*.

When D.D.T. is applied to surfaces in solutions or emulsions, it has frequently been noted that there is a pronounced tendency for supersaturated solutions or supercooled liquid to be formed on evaporation of the solvents (Parkin & Green, 1945, 1947; Patten & Sarkaria, 1948). The residues may later crystallize to give solid films. Similarly, D.D.T. incorporated in paints, lacquers or plastics, slowly crystallizes from the film that is at first obtained (Block, 1948*a*, *b*; Gilmour, 1946). Parkin & Green (1945) were the first to note that this crystallization is accompanied by a marked increase in toxicity of the films; the later workers (Block, Gilmour) have confirmed this rather peculiar finding.

Hadaway & Barlow (1947) have reported that D.D.T. dispersible powders gave much more efficient films than emulsions or solutions on mud walls. This is attributed to the fact that D.D.T. in solution penetrates some distance into the mud and is consequently not available to alighting insects; the relatively large D.D.T. crystals in dispersible powders are retained on the surface of the wall.

Although all this work by other authors was carried out with one kind of residual film or another, the point common to the present and other work is the fact that relatively *massive* D.D.T. crystals can be highly toxic to insects. This seems to be quite a general finding as it applies to houseflies (Block, 1948*a*, *b*; Gilmour, 1946; Parkin & Green, 1945, 1947; Vickers, 1946), red-scale crawlers (Ebeling, 1945),

tsetse flies and *Aëdes aegypti* (Hadaway & Barlow, 1947), as well as to *Tribolium castaneum* and *Oryzaephilus surinamensis*.

It is by now well known that D.D.T., in the form of an aqueous colloidal suspension, is extremely effective as a mosquito larvicide (e.g. Deonier, Maple, Jones, Hinchley & Eide, 1945; Jones & Fluno, 1946); the results obtained here against grain beetles show that colloidal D.D.T. is the least effective form. However, it must be remembered that the two methods of application are very different. The mosquito larvae, being aquatic, are likely to remain in contact with suspensions for a matter of days, and in consequence of this and of their method of feeding, stomach action, besides cuticular entry, is inevitable. Two species as different as adult grain beetles and mosquito larvae might at all events be expected to respond in different ways to variations in particle size. Further, anything other than a colloidal suspension of D.D.T. would be of small value as a larvicide, as the particles must remain suspended for a considerable period of time, and must be small enough to enter the mouth parts of the larvae.

With rotenone, an inverse relation of toxicity to particle size (the opposite effect to D.D.T.) has, as stated, been long suspected, and confirmed by the present work. As the previous workers used a variety of insect species, namely silkworm (Fryer *et al.* 1923), red-spider mite (Richardson, 1935), *Ahasverus advena* (Cahn *et al.* 1938), and *Aphis rumicis* (Tattersfield & Martin, 1938), it would also seem that the results noted here on *Oryzaephilus surinamensis* are of quite general application.

There is no evidence, in the present work, of any optimum rotenone crystal size, nor that crystal shape is of importance. It seems that, with crystalline suspensions, differences in crystal form must be fairly large before toxicity differences can be noted. The differences in shape amongst types III, IV and V may not be great enough to influence potency.

It is to be expected that, by analogy with D.D.T., more rotenone would be retained by insects from coarse suspensions than from equitoxic or even equal concentrations of fine suspensions. It is not, in fact, necessary to carry out chemical retention analyses to establish this point, for at the time of inspection of *O. surinamensis* treated with higher concentrations (*c.* 0·2%) of large hexagons, the insects (including survivors) are frequently found to be covered with a visible layer of crystals. No residue is ever to be seen on insects treated with colloidal rotenone, which gives 100% kill at concentrations some 200 times lower.

In our experiments, approximately the same range of sizes has been covered with D.D.T. and rotenone, but exactly opposite results have been obtained. Thus, increasing toxicity of D.D.T. suspensions is associated with increasing size of particle in suspension, and with rotenone, maximum toxicity is found with the smallest particles. In addition, rotenone shows a much greater response in median lethal concentration (*c.* 600 times) than D.D.T. (*c.* 20 times), to variations in particle size. In both cases the least toxic form gives the flattest probit line.

It would appear that treatment with coarser suspensions of both D.D.T. and

rotenone results in the actual application of a higher dosage of poison to the insects. With rotenone, this is not accompanied by a higher kill. On the other hand, with D.D.T. the increased dose can apparently be utilized by the insects. An attempt at an explanation of how these differences come about, and of the time-mortality results, will be published in a later paper.

The author wishes to express his thanks to Dr F. Tattersfield and Dr C. Potter for their guidance and suggestions, and to Mr V. Stansfield for preparing the photomicrographs.

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EXPLANATION OF PLATE 13

Fig. 1. Large hexagons. ×80.
Fig. 2. Small elongated plates. ×420.
Fig. 3. Small hexagons. ×420.
Fig. 4. Hexagon aggregates. ×420.

(*Received* 9 *April* 1949)

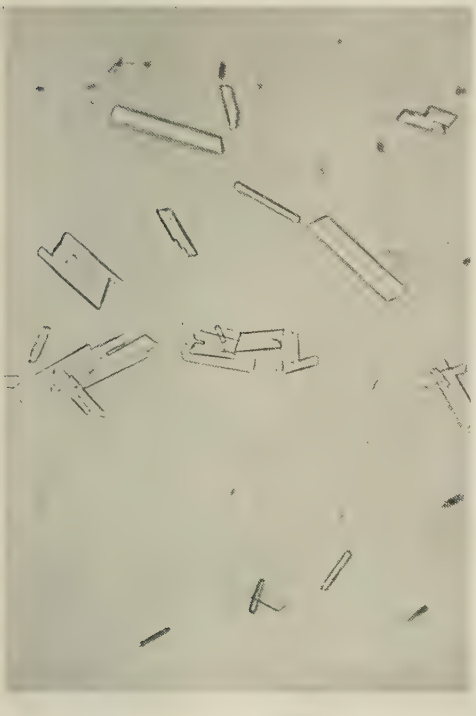

Fig. 2

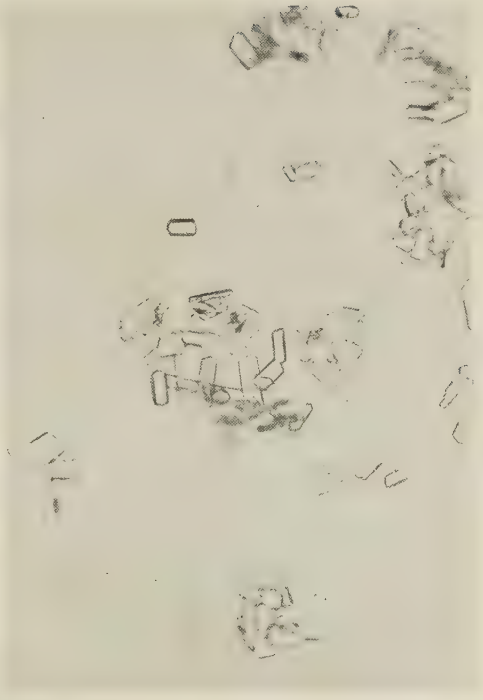

Fig. 4

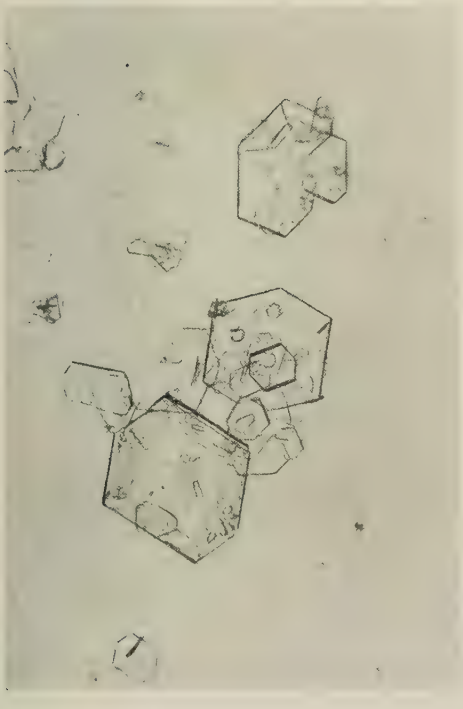

Fig. 1

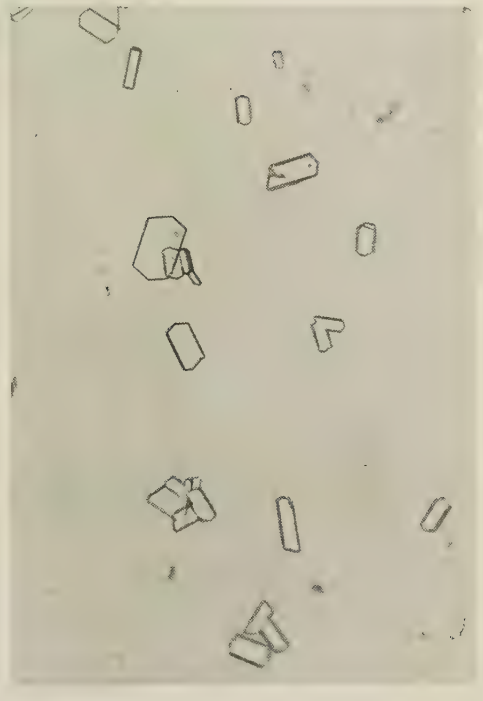

Fig. 3

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Wednesday, 16 March 1949, in the Imperial College of Science and Technology, London; the President, Mr G. Fox-Wilson, in the Chair.

Growth-promoting Substances in Agriculture and Horticulture

After an introductory talk by Prof. F. G. Gregory, the following papers were read and discussed:

1. Attempts to produce parthenocarpic pears with growth substances. By Miss D. J. Osborne.

2. The use of growth substances to control the shedding of fruit. By Mr M. C. Vyvyan.

3. Chemical aspects of plant growth-regulating activity. By Dr R. L. Wain.

4. The use of growth-promoting substances in the vegetative propagation of plants. By Dr E. S. J. Hatcher.

5. Fruit-drop in the apple in relation to seed development. By Dr L. C. Luckwill.

ATTEMPTS TO PRODUCE PARTHENOCARPIC PEARS WITH GROWTH SUBSTANCES

By Daphne J. Osborne, *Wye College, University of London*

The production of parthenocarpic pears by synthetic growth substances has been described by Russian and Dutch workers. Sereisky (1938) removed ovules from small fruits of *Pyrus communis caucasus* and replaced them with a lanolin paste containing 0·05% phenylacetic acid. He also placed lanolin plus 0·5 or 0·1% heteroauxin, and lanolin plus 0·1% phenyl-acetic acid on the cut end of the styles of flowers that had had stamens and stigmas removed. A set of pears not exceeding 30% was obtained from the treatments. Van Stuivenberg (1943), at Wageningen, replaced the ovary cavities of frosted blossoms of Precoce de Trevoux with lanolin paste containing a mixture of β-indolyl-acetic acid and potassium naphthyl-acetate, and obtained a 42·1% set of parthenocarpic fruit.

At Wye College, in 1947, compounds known to be effective in the setting of parthenocarpic fruit in tomatoes were sprayed on to freshly emasculated pear blossom of the varieties Dr Jules Guyot and Beurre Superfin. Petals, stamens, styles and sepals were removed by cutting the blossom across beneath the sepals with a sharp pair of scissors. A wide range of compounds was sprayed on to the fruits, each being made up as the sodium salt with a suitable setting agent incorporated. After 14 days, it was evident that α-(2-naphthoxy)-propionic acid was far more effective than all other compounds used. With this treatment,

twenty-four out of thirty-one emasculated blossoms developed on Dr Jules Guyot and nineteen out of twenty-eight on Beurre Superfin. The maximum diameter of these fruits was, for 24 days, larger than that of normally fertilized controls. Thus a single application of α-(2-naphthoxy)-propionic acid, in addition to inducing good set, stimulated early growth of the fruitlets. Subsequently, however, development ceased, suggesting that the original application of growth substance had proved insufficient to carry the young pears to maturity. Luckwill (1948) has produced evidence that a continuous supply of hormone from the endosperm of young fertilized apple pips may be correlated with fruit development. Accordingly, one truss of pears that had received treatment was resprayed with solution at the same concentration as before. This respray treatment did not, however, initiate any further growth, and all the blossoms abscissed within a few days. This second application of growth substance was almost certainly made too late, growth having ceased completely. If the pears had received a continuous supply of growth substance in the early stages of enlargement, growth might well have continued until maturity.

In 1948, a full-scale experiment was set out on pear varieties Pitmaston Duchess and Dr Jules Guyot, using α-(2-naphthoxy)-propionic acid at different concentrations. Blossoms were emasculated at the white-bud stage, but by a more refined technique than in the previous year. The petals, stamens and stigmas were removed with a sharp scalpel, so involving the minimum of damage. In all, some 750 blossoms were treated.

Following the initial spray, which induced development as before, four methods were employed for supplying a continuous source of growth substance to the young pears in their early stages of growth. These were as follows:

(1) By applying the growth substance in a lanolin paste (at 0·5%) to the pedicel or receptacle of the young fruitlet, and thus making available a source of growth substance over a prolonged period.

(2) By spraying the fruitlets every 3 days with an aqueous solution of the growth substance, at 100 p.p.m.

(3) By injection of an aqueous solution of growth substance into the ovary by means of a fine microhypodermic syringe.

(4) By feeding the young fruitlets with an aqueous solution of the compound by immersing the midrib of the subtending leaf in a tiny tube containing the solution.

Of these methods, the first two proved successful. The lanolin treatment gave a response only on the Dr Jules Guyot variety; a 25% set of parthenocarpic fruit was obtained.

The most effective responses on Pitmaston Duchess were obtained on trusses receiving the single spray at 250 p.p.m. and those originally sprayed with 100 p.p.m. of the compound and resprayed at 3-day intervals. In both these experiments the pears stayed on much longer than the majority of the emasculated water-and-wetter sprayed controls, and the highest proportion reached maturity, 25% with the 250 p.p.m. spray, and 37·5% with the respray treatment. A small proportion (12·5%) of the water-and-wetter sprayed controls also reached maturity, and this is accounted for by the small degree of parthenocarpy normal to this variety of pear. Measurements of the mean diameters of fruits on selected trusses of each treatment showed that for about 6 weeks the largest fruits were those sprayed every 3 days, the smallest being the water-and-wetter sprayed and the normally fertilized controls. Respray treatments using α-napthyl-acetic acid and β-indolyl-acetic acid were completely ineffective.

The variety Dr Jules Guyot appears to be more sensitive to treatment, since the highest proportion (30·5%) of mature fruits was obtained with a single spray α-(2-naphthoxy)-propionic acid at 100 p.p.m. The 3-day respray treatments were effective in preventing abscission until the fruit was nearly half-grown, after which all fruits dropped within a period of 20 days, an effect probably due to overtreatment. In this variety, too, 12·5% of emasculated water-and-wetter sprayed controls reached maturity.

In the large-scale 1949 experiments now in progress, the same compound has been applied to the varieties treated in 1948. On trees of Dr Jules Guyot, no set whatever has been

achieved from emasculated blossoms which have been left unsprayed, or which have received water or water-and-wetter. On the other hand, not a single blossom of the 175 sprayed with α-(2-naphthoxy)-propionic acid at 100 p.p.m. failed to set. Further, all fruitlets which have received five or more sprays are still developing and many should reach maturity. It is possible that the small set of blossoms recorded in this variety following water-and-wetter treatments in 1948, was in some way due to the use of the same trees for different treatments. In 1949, the effect of spray drift, and possible translocation effects have been completely avoided by using separate trees for the different experiments.

The parthenocarpic fruits of Pitmaston Duchess produced by α-(2-naphthoxy)-propionic acid appeared quite normal, while those of Dr Jules Guyot were somewhat smaller than normally fertilized fruits and rather plum-shaped. The pips from the parthenocarpic fruits from both varieties were small and flattened, and contained no embryos.

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THE USE OF GROWTH SUBSTANCES TO CONTROL THE SHEDDING OF FRUIT

By M. C. Vyvyan, *East Malling Research Station, Kent*

Normal losses of blossoms and fruits

In most fruit trees, especially the apples and pears, only a small proportion of the blossoms survive to become mature fruits. Most are shed at some intermediate stage by the development of abscission layers at the bases of the fruit stalks. Those that become ripe are ultimately cast off by the same means, if they have not been picked. This shedding, or fruit drop, tends to occur in waves which vary somewhat with the variety, locality and season.

The first wave, sometimes called the 'first drop', occurs shortly after petal-fall and often consists largely of unpollinated or unfertilized blossoms. Fruits that survive this drop usually remain on the tree for several weeks, increasing in size more or less exponentially, largely by cell division, and presumably becoming an ever-growing strain on the resources of the tree.

The second wave, commonly called the 'June drop', tends to occur when the fruits are about the size of walnuts; its severity may be inversely proportional to that of the 'first drop' and directly proportional to the relative number of surviving fruits.

In some varieties, in some places and seasons, there is little further drop until the fruits are ripe or over-ripe. In others, such as Bramley's Seedling, there may be a slow but steady drop of fruits up to picking time, resulting in a considerable cumulative loss of crop. Others may hold their fruit until they are nearly mature, and then suddenly shed most of the crop in a 'pre-harvest drop'.

Adjustment of the crop to size of tree

The early shedding of blossoms and fruits is usually an advantage, as there are normally far too many of these on the tree. If all were retained, they would remain small and poor in quality and there would be a danger of broken branches. Moreover, their presence during

the critical period for fruit-bud initiation might restrict this process and reduce the number of blossoms for the following year. If too few are shed naturally, they have to be removed artificially, and this thinning process is an expensive operation. The converse, however, also holds true. If too few fruits survive, these tend to become individually too large and of poor quality, and excessive fruit-bud initiation may result in an 'on' year next season, and the tree may be thrown into a biennial rhythm of alternate heavy and light crops. This often happens when a frost kills all the blossom or young fruits.

Thus the early drop must be neither too light nor too severe if the surviving crop is to be the correct size for good quality and regular bearing. In thinning we have a useful tool for supplementing the natural drop when this is too light; what is required is a complementary tool for reducing drop when it tends to be too severe.

Pre-harvest drop of nearly mature fruits has probably little effect on quality or regularity in cropping, and is thus largely an unredeemed loss; its control is therefore very desirable.

Some control of drop can be achieved by cultural methods, such as grassing down or the planting of cover crops. Breeding of varieties with a natural power of self-adjustment is another possibility. These methods may be useful as a long-term policy, but they do not meet the needs of a sudden emergency.

Use of sprays of α-naphthalene-acetic acid, or its derivatives, to control the pre-harvest drop

A rather effective tool for the control of pre-harvest drop in some varieties has been found in the use of certain synthetic growth substances; sprays containing these are now being used on hundreds of thousands of acres of fruit trees in the U.S.A. and elsewhere. The forging of this useful tool was largely due to the American authors, Gardner, Marth & Batjer (1939). They noticed that the abscission of leaf petioles on cuttings was sometimes postponed when the cuttings had been treated with certain growth substances to promote rooting, so they carried out experiments to see if these substances would delay abscission on fruit stalks also. They found that sprays containing α-naphthalene-acetic acid, its salts, or the related acetamide, were very effective in controlling pre-harvest drop in certain varieties of apple when used at concentrations of 10 p.p.m. or less. Indole derivatives were relatively ineffective. They found that the actual fruit stalk must be wetted by the spray, and that long-stalked varieties and those that matured early in the season responded best. The duration of the effect varied from some 10 days in the case of one variety—McIntosh—to some 4 weeks in the case of others. It was only the pre-harvest drop they were able to control; early sprays failed to reduce the June drop. As a result of this work, and of numerous experiments that were immediately carried out on the same lines in the U.S.A. and elsewhere, many proprietary products were put on the market and have become widely used. Use of the sprays has been extended to pears, but stone fruits in general have not responded well.

East Malling got early notification of this work and started experiments on the same subject in 1940 (Vyvyan, 1941) to determine whether similar results could be obtained with English varieties. The results have been published (Vyvyan, 1946*a*; Vyvyan & Barlow, 1947, 1948*a*, *b*) and a fairly comprehensive review of all the work published up till 1946 has also appeared (Vyvyan, 1946*b*). All that there is time for here is a brief summary of some of the main results achieved with a few English varieties. This will serve as an illustration of the increase in crop that can be achieved by these sprays.

The results of four experiments on the early-maturing apple variety Beauty of Bath are shown in Table 1; three were carried out at East Malling, the other by Swarbrick (1945) at Long Ashton. The crop was increased in some cases threefold, in others eightfold. Some of the drops from the unsprayed trees, however, would have been marketable.

The results of five experiments on the mid-season apple variety Worcester Pearmain are shown in Table 2; again one experiment was at Long Ashton. The increase in crop was again considerable and of economic importance. Growers often have a large acreage of this variety

TABLE 1. *Variety Beauty of Bath. Control of pre-harvest drop by sprays of α-naphthalene-acetic acid (N.A.A.) at East Malling (E.M.) and Long Ashton (L.A.)*

Place and year	Percentage picked			Gain in bushels/acre
	−N.A.A.	+N.A.A.	Diff.	
E.M. 1940	11	33	**22**	26
E.M. 1945	9	77	**68**	293
E.M. 1945	31	91	**60**	295
L.A. 1944	10	82	**72**	—

TABLE 2. *Variety Worcester Pearmain. Control of pre-harvest drop by sprays of α-naphthalene-acetic acid (N.A.A.) at East Malling (E.M.) and Long Ashton (L.A.)*

Place and year	Percentage picked			Gain in bushels/acre
	−N.A.A.	+N.A.A.	Diff.	
E.M. 1940	86	94	**8**	34
E.M. 1943	56	87	**31**	187
E.M. 1945	41	77	**36**	254
E.M. 1946	9	50	**41**	309
L.A. 1944	20	55	**35**	—

and have to spread the harvest over several weeks. In such cases it is useful to spray the orchards that are to be picked last, otherwise they may develop a heavy drop. Good results have also been obtained with the apple variety Miller's Seedling, the pear Conference, and, in 1948, with the important apple variety Cox's Orange Pippin, when picking was postponed for a fortnight. Several varieties have not responded well; these include Bramley's Seedling at East Malling and Edward VII at Long Ashton.

To control June drop

Gardner *et al.* (1939) failed to control June drop and few further attempts seem to have been made, partly, perhaps, because this drop is often of value. Early thinning operations, however, could be carried out with greater confidence and precision if subsequent drop could be prevented. Cox's Orange Pippin is a variety that often suffers from an excessive loss of fruit at this period or a little later. Attempts at controlling this drop with sprays of α-naphthalene-acetic acid (Vyvyan & Barlow, 1947, 1948*a*) are shown in Table 3. Very considerable control was obtained in both years, whether the growth substance was used alone or added to a routine spray such as lead arsenate. These sprays sometimes continued to exert a controlling influence on drop for several months.

TABLE 3. *Variety Cox's Orange Pippin. Control of June drop by α-naphthalene-acetic acid (N.A.A.). Numbers picked, as percentages of numbers on the trees, at the time of spraying*

Date of spraying	Insecticide or fungicide	Growth substance	
		None	N.A.A.
1 July 1946	None	12	44
19 June 1947	None	12	19
	Lead arsenate	27	36
18 June 1947	None	15	25
	Lead arsenate	22	30
	Dispersible sulphur	17	26
	Summer petroleum	16	19

Possible improvements in the control of fruit drop by the use of other growth substances

Where sprays of α-naphthalene-acetic acid fail to give adequate control, the use of other growth substances is worth a trial. The only ones so far tried on a considerable scale are 2, 4-dichloro-phenoxy-acetic acid and its derivatives. These have been found effective in the U.S.A. with two apple varieties, Winesap and Stayman (Batjer & Marth, 1945; Batjer & Thompson, 1946, 1947), but not with others. The acid had some effect on Bramley's at East Malling but not with Cox's (Vyvyan & Barlow, 1947, 1948*a*, *b*). These substances have also been effective with the pear Williams' Bon Chrétien (Bartlett) (Batjer, Thompson & Gerhardt, 1948) and with grape fruit (Stewart & Parker, 1947). Unfortunately, these substances often cause damage both to the fruit and to the tree, especially if the butyl esters are used (Harley, Moon & Regeimbal, 1947), and the effects tend to persist and to cause damage, or even a reduction in crop, in the following year (Moon, Regeimbal & Harley, 1948). Some other growth substances have been tried, usually on a small scale and with little success; these, and others not yet tested, should be tried on a larger scale. It is impracticable, however, to use the whole tree as an experimental unit when comparing large numbers of different substances. A new technique is therefore being developed at East Malling (Barlow, 1948) in which the single leaf or fruit stalk is used as a unit.

Methods of application

Development of new methods of application is another possible way of improving control of drop. Water sprays are the usual and safest method; dusting is sometimes convenient but often gives poor results. Aerosol methods have been tried, and high concentrations of naphthalene-acetic acid, for example, 2400 p.p.m. in a 40% oil emulsion applied at the rate of 5 gal./acre, are now being applied in this form on a large scale by aeroplane in the U.S.A., presumably with satisfactory results. There are indications, however, that many fruiting spurs are missed by this method, and that there is no transmission of effect from spur to spur (Batjer & Thompson, 1948). Moreover, there is always danger in this method when dealing with a substance that is liable to cause damage when used at a high concentration.

The injection method has been successful in a recent small-scale experiment with 2, 4-dichloro-phenoxy-acetic acid (Edgerton & Hoffman, 1948).

Effect on maturity and behaviour of fruits in store

Sprays containing α-naphthalene-acetic acid have sometimes hastened maturity in certain early apple varieties in the U.S.A. but have had no apparent effect on mid-season or late varieties, provided these have been picked at the proper time (Batjer & Moon, 1945). When picking has been postponed, the sprays have sometimes hastened maturity in certain apple varieties and the Williams' Bon Chrétien pear (Gerhardt & Allmendinger, 1946). Trials at the Ditton Laboratory by Dr West and his colleagues, working in collaboration with East Malling Research Station, have shown that sprays of α-naphthalene-acetic acid have had no effect on the storage behaviour of fruits of Bramley's Seedling, picked at the normal time, or those of the apple variety Barnack Beauty and the pear Conference, even when picking has been postponed. Delay in picking, as such, had a large effect (Vyvyan, West & Barlow, 1948). Similar results have since been obtained with Cox's Orange Pippin.

Damage to fruit or tree

There seems little evidence that sprays of α-naphthalene-acetic acid, used at concentrations of 10 p.p.m. or under, have ever caused any serious damage to fruits or tree. Many instances of damage by 2, 4-dichloro-phenoxy-acetic acid sprays have been reported, as mentioned above, and use of such sprays seems unwise at present.

Investigation of processes underlying fruit drop and its control

At present we have little real knowledge about the nature of the abscission process or of the chemical reactions involved. Fuller knowledge about these would be likely to yield useful results.

Luckwill's (1948) recent demonstration of the production of a hormone by the endosperm of apple seeds and its relation to fruit drop seems to be the first real direct observation that a natural hormone controls abscission. Hitherto all evidence has been indirect, though fairly conclusive. There is still the problem of how the hormone achieves its effect; Portheim (1941) suggested that it was through its influence on water relations, but there is evidence that abscission may occur under conditions of low and high moisture. An important problem of even wider nature is that of the mode of action of synthetic growth substances. Why do these substances, which apparently do not occur naturally in the plant, produce effects similar to those induced by natural hormones? Do they take their place and perform the same function, or do they merely influence the formation, movement or activation of natural hormones in the plant?

Summary

Sprays containing α-naphthalene-acetic acid, its salts or acetamide, often reduce pre-harvest drop in many varieties of apple and pear, and the June drop of Cox's Orange Pippin.

Such sprays have sometimes hastened maturity of early apple varieties in the U.S.A. but have not affected storage behaviour of the apples Bramley's Seedling, Barnack Beauty, Cox's Orange Pippin or the pear Conference in England.

Sprays containing 2, 4-dichloro-phenoxy-acetic acid have sometimes given good control of drop, but have proved dangerous to the tree. Effect of other growth substances and other methods of application needs to be examined.

The processes underlying abscission, its control by natural hormones, and the mode of action of synthetic growth substances, require fuller investigation.

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CHEMICAL ASPECTS OF PLANT GROWTH-REGULATING ACTIVITY

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In 1934 Kögl and his collaborators at Utrecht isolated β-indolyl-acetic acid from human urine and showed that this compound was markedly active in promoting cell elongation in plants. This important discovery, which revealed that a comparatively simple organic molecule could possess growth-regulating properties, led not only to attempts to improve the activity of β-indolyl-acetic acid by introducing substituent groups and other means, but stimulated a search for active materials outside the indole series. At the present time a large number of compounds which possess growth-regulating properties are known. These range in complexity from simple molecules like carbon monoxide and ethylene to complex substances like the natural auxins.

Zimmerman and Hitchcock of the Boyce-Thompson Institute have been prominent workers in this field. It was they who, in 1933, demonstrated the activity of carbon monoxide, acetylene, ethylene and propylene and who, in 1935, showed that phenyl-, α- and β-naphtha-

lene-, anthracene-, acenaphthene- and fluorene-acetic acids all possessed growth-regulating activity.

Following the discovery by Irvine (1938) that β-naphthoxy-acetic acid possessed activity, Zimmerman and Hitchcock examined a large number of substituted phenoxy and naphthoxy acids, many of which were found to be highly active. Other types of compound are now known to influence plant growth; for instance, traumatic acid (Δ′-decene-1-10-dicarboxylic acid) possesses 'wound hormone' properties, and various esters of phenyl carbamic acid may profoundly affect the growth of grasses and cereals.

In this short paper I intend to restrict myself, in the main, to substances of the phenoxy and naphthoxy acid type. There is now a great deal of evidence on which to base considerations of chemical structure in relation to activity. All such work, however, depends on an accurate assessment of growth-regulating activity, and the available methods by which individual compounds may be compared are important.

The methods which have been most widely used are: (*a*) *Avena* test (depends on measurement of curvature induced in a decapitated oat coleoptile); (*b*) oat cylinder test (depends on the measurement of growth of cylindrical pieces of oat coleoptile immersed in a solution of growth substance); (*c*) Went pea test (depends on the measurement of curvature induced in divided pea shoots following immersion in a solution of growth substance). In addition, other tests are available amongst which those based on the capacity to promote rooting of cuttings or capacity to promote parthenocarpic development of fruit may be mentioned.

It is often found that variation in activity is shown by a compound when examined by different tests. For example, Thimann has shown that indene-acetic acid is only very slightly active in the *Avena* test, is more active in the oat cylinder test and even more so in the Went pea test. Similarly, Miss Osborne has found α-naphthoxy-acetic acid and α-(1-naphthoxy)-*n*-butyric acid to be slightly active in the pea test but inactive in the cylinder test.

Such observations have led Thimann to conclude that observed activity may depend on a number of factors. Not only must the compound possess inherent growth-regulating activity but it must possess the necessary physical properties to reach the site of action and it must not be deactivated *en route*. Thus a compound with low penetrative properties might be expected to show low activity in the *Avena* test simply because little of the compound reaches the growing cells. A good example of this is provided by the *dextro* and *laevo* forms of α-(indole-3)-propionic acid. Kögl (1937) showed that, in the *Avena* test, the *d*-form of this acid is thirty times more active than its stereoisomer. The latter, however, was shown by Verkaaik (1942) to be absorbed by cell tissue to a much greater extent than the *d*-form. It would appear, therefore, that more of the *d*-acid reaches the site of action, the difference being one of transport. In the pea test, however, where the plant material is actually immersed in the solution and penetration thereby greatly facilitated, more of the material should reach the site of action and, if the compound is active, a response should be shown. Growth-regulating activity is therefore difficult to assess and the danger of using the *Avena* test alone is obvious.

Turning now to chemical aspects, following the well-known procedure used in the field of chemotherapy, synthetic insecticides and so on, many attempts have been made to improve the activity of a growth substance by modification of its chemical structure. By such methods as introducing further groupings into the molecule, reduction of double bonds and so on, activity is usually affected. Indeed, highly active materials such as 2:4-D have emerged from such investigations.

Whether or not such research will eventually reveal clear relationships between chemical structure and activity, and perhaps lead to an understanding of mode of action, is open to question, for this so-called activity may embrace a most complicated series of reactions which are, of course, not necessarily chemical. Certainly, very few such simple relationships between structure and activity have emerged in other related fields of work. Nevertheless, such research is of undoubted value and may provide a short cut to the discovery of other highly active compounds or provide fundamental knowledge on mode of action. In the field

of chlorinated synthetic insecticides, the theory on the mode of action of D.D.T. put forward by Dr H. Martin and myself and which was based on research of this type, has led directly to the discovery in America of Chlordan and other highly insecticidal derivatives.

Now let us turn to the growth-regulating substances of the substituted acetic acid type and see what has emerged from the researches carried out to date. Koepfli, Thimann & Went (1938), after examining a range of such compounds by the pea test, gave as minimum requirements for activity: a ring system containing at least one double bond with a side chain carrying a carboxyl (COOH) group (or grouping which may readily be converted into a COOH group), there being at least one carbon atom between the ring and the COOH group. Also, there must be a particular space relationship between the ring and the COOH group. What is the evidence at the present time for these assumptions? Firstly, saturated and unsaturated open-chain carboxylic acids which have been examined are inactive, as are compounds of the type $CH_3(CH_2)_nCH_2.O.CH_2COOH$, recently investigated at Wye. Veldstra (1947) has found trichloroacetic acid to possess slight activity in the pea test. In general, however, the presence of a ring system may be regarded as necessary. Further, all such active compounds contain at least one double bond. Thus, for instance, if the double bond in the cyclopentene nucleus of auxin *a* or *b* is reduced, activity is destroyed and in compounds containing more than one double bond in the ring system, reduction of these one at a time usually reduces activity.

The function of the double bonds is not apparently to confer easy reducibility and enable the molecule to take part in oxidation-reduction processes. Veldstra (1944*b*) is rather of the opinion that the function of the ring system is to increase surface active properties and to facilitate orientation of the molecule when adsorbed at a sensitive interface. He points out that the number and arrangement of the double bonds may exert an important influence.

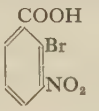
Turning now to the side chain, the first point to observe is that the position at which this side chain carrying a COOH group is attached to the ring can greatly influence activity. Thus, indole-3-acetic acid is very active whereas indole-2-acetic acid possesses low activity. Similarly, α-naphthyl-acetic acid has high and the β-derivative low activity whereas β-naphthoxy-acetic acid has high and the α-acid low activity.

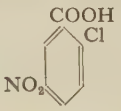
In addition to the carboxylic acids, esters, amides and nitriles may possess growth-regulating properties. All these groupings can form —COOH by reaction with water, but whether the groupings themselves confer activity has not been proved. α-naphthyl aceto-nitrile, however, is inactive in the pea test—a test of short duration, although this compound is highly active in promoting root formation—a process in which there is certainly more time for the hydrolysis of the —CN group to —COOH to take place.

Veldstra (1944*b*) has studied the effect of substituting other groups for carboxyl in the α-naphthyl-acetic acid molecule. The groupings —Cl, $—CH_2OH$, $—NH_2$ and $—SO_3H$ all gave products which were inactive in the pea test. The —CHO group, however, gave an active product and the $—NO_2$ group, a product of slight activity. At Wye, we have prepared and studied compounds of the type  —OCH_2SO_3Na and found them to possess negligible activity.

It would therefore appear that in practically all active compounds the carboxyl group has a specific effect.

Much work has been carried out on the influence on activity of increasing the length of the side chain. It will be remembered that Koepfli *et al.* stated that the —COOH group must be separated from the ring by at least one carbon atom. This condition is fulfilled by most highly active compounds, though Zimmerman and Hitchcock have reported 2-bromo-3-



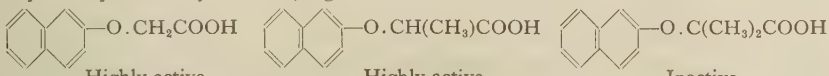


nitro- and 2-chloro-5-nitro-benzoic acids to be effective in promoting cell elongation. In these compounds, of course, there is no bridging group between the ring and the carboxyl grouping.

With side chains containing more than two carbon atoms, interesting effects are observed. Thus, Grace (1939), studying higher homologues of α-naphthyl-acetic acid, found that the capacity to promote rooting was less in compounds with an odd number of carbon atoms in the side chain (e.g. propionic, valeric) than those like α-naphthyl-acetic and butyric acids with an even number. A similar effect in the β-indolyl-acetic, propionic and butyric acid series was reported by Thimann & Bonner (1938). On the other hand, with the α-substituted propionic acid derivatives, which are, in effect, substituted acetic acids, high activity is usually shown.

Synerholm & Zimmerman (1947) have prepared the homologues of 2:4-dichlorophenoxy-acetic acid up to the caprylic acid (C_8) derivative and demonstrated that activity is shown only by those acids which contain an even number of carbon atoms in the side chain. These findings were explained by the suggestion that β-oxidation operates a mechanism by which only those acids possessing an even number of carbon atoms in the side chain would be converted to the active oxy-acetic derivative.

The effect on activity of replacing the oxygen bridge in β-naphthoxy-acetic acid by S and by NH has been studied at Wye. Both compounds showed negligible activity. We have also been concerned with the effect on activity of substitution in the side chain of certain phenoxy- and naphthoxy-acetic acids. In all series examined, substitution in the position of one methyl group usually has little effect on activity but when two methyl groups are introduced, activity is very markedly reduced, e.g.



Highly active Highly active Inactive

It is interesting to note that phenyl-acetic and α-phenyl-propionic acids are active, whereas phenyl-*iso*-butyric acid is inactive when examined by the pea test. The reason for this sudden drop in activity when the α-carbon atom is fully substituted is obscure. We are of the opinion that for good activity at least one hydrogen atom must be carried by the α-carbon atom and we do not rule out the possibility that the function of this hydrogen is to facilitate chemical reaction. In this connexion Veldstra's α-naphthyl-nitro-methane is of interest, for here he postulated that the *aci* form is active, a form which can be reached only by the migration of a hydrogen atom from the α-carbon.

We now come to the last requirement of Koepfli *et al.*, that there must be a definite special arrangement between the ring and the carboxyl group. Veldstra (1944*a*) has produced evidence to show that for activity the dipole of the carboxyl group should not be in the same plane as the ring, the ideal position being reached when the two are perpendicular to each other. In support of this suggestion, Veldstra quotes the cases of the two forms of cinnamic acid where only the *cis* form is active. In this form the COOH group is out of the plane of the ring whereas in the *trans* it is not.

I have so far said nothing about the effect of substitution in the nucleus on growth-regulating activity. That such substitution may have a marked influence is shown, for example, in the series: Phenoxy-acetic acid (almost inactive), *o*-chlorophenoxy-acetic acid (active), *p*-chlorophenoxy-acetic acid (more active), 2:4-dichlorophenoxy-acetic acid (highly active). Although there are definite indications that certain types of nuclear substitution may increase or decrease activity, no general conclusions can yet be made.

Though the site of action of growth substances has not been fully established, Veldstra (1944*b*) has indicated that the chemical becomes adsorbed at, and influences the properties of, the protoplasmic membrane. He concludes that the ring system largely determines the degree to which adsorption occurs and that the growth-regulating activity proper is dependent on the carboxyl group, which must however be in a specific spatial relationship to the ring.

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THE USE OF GROWTH-PROMOTING SUBSTANCES IN THE VEGETATIVE PROPAGATION OF PLANTS

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INTRODUCTION

Compared with the centuries-old art of the plant propagator the use of growth substances to promote rooting is very recent. Only perennial plants naturally propagate their species vegetatively, though annuals and biennials can be artificially increased in this manner. In nature the new individual is formed before severance from the parent plant, whereas in artificial propagation this is often not the case. In natural regeneration, e.g. the strawberry runner or the tip of the bramble shoot, the processes leading to root formation begin when a suitable environment is reached, namely, slight penetration of the soil. The propagator, on the other hand, has a range of techniques at his disposal extending from those akin to the natural methods, such as layering and stooling, through intermediate types, to the cutting proper in which the regeneration process occurs wholly after separation.

In layering, aerial shoots are pegged along the soil and lightly covered; as shoots grow up from the buds they are induced to develop roots at their base by mounding up the soil. In stooling the parent plants are pruned back hard and the new shoots arising are induced to produce roots by earthing up. As in the natural methods, the rooting process in both methods occurs before separation from the parents.

In the cutting method the shoot is first removed and then propagated. Both preparatory and initiatory processes must therefore take place after removal, and this institutes the inherent difficulty of the technique. The base of the cutting is buried either out of doors or in a propagating frame, thus providing the conditions essential to rooting, but, of course, subsequently to separation. There are, however, two examples of cutting propagation which may be regarded as intermediate (i) in those stems where root initials commonly occur giving the cutting a potential start, e.g. *Ribes*, *Salix* (these tend to be easy rooters) and (ii) cuttings taken from the etiolated base of a layer or stool shoot, where even if no roots have actually appeared the predisposition to root formation is strong.

The development of the growth-substance method

It was in cutting propagation that horticulture first used synthetic growth-promoting substances, a use anticipated by Sachs more than seventy years ago. According to Sachs the isolation of the piece of stem was followed by the accumulation of special substances at the base, as a result of which roots were initiated. These substances, he postulated, occurred in amounts too small for ordinary chemical analysis, and displayed polar movement in the plant tissues. How prophetic was this view has been demonstrated by events leading to the isolation of the auxins.

The key discovery was the identification of heteroauxin as indole-3-acetic acid, for it meant the beginning of widespread experimentation on the effect of applying a pure growth substance to the plant externally; and it led immediately to chemical synthesis of similar substances and the exploration of the relationship between molecular structure and physiological activity.

Regarding root formation by the cutting it soon became evident that the addition of an external auxin supply did have an added effect to any produced by naturally occurring substances, and hundreds of varieties and species have been found to respond; in root initiation, in acceleration of the rooting process and in the greater production of roots. One may quote the surveys of Pearse (1948), Avery & Johnson (1947) and Thimann & Behnke (1947), all producing lengthy tables of results illustrating the undoubted efficacy of the growth-promoting substance. Nevertheless, certain plants usually failed to respond and a tradition grew that growth substances could aid rooting in those cases where it was able to take place without external stimulation, but could not do what the propagator really wanted, namely, establish plants from cuttings of species and varieties not ordinarily capable of doing so. Because of this, growth substances have been in danger of ill repute, despite their undoubted potentialities, and it is salutary to examine their effect in relation to all the factors operating in the rooting process.

Technique of growth-substance treatment

Of many substances synthesized and examined, two are outstanding as greatly superior to the natural auxin indole-3-acetic acid,

(i) indole-3-butyric acid; and

(ii) naphthyl-1-acetic acid (more commonly α-naphthalene-acetic acid).

Their behaviour is associated with two important characteristics; (*a*) they are much more stable than indole-3-acetic acid, and (*b*) they are not so readily transported in the plant.

Application to the cutting has been made in various ways:

(i) In *lanolin*, almost always an experimental technique.

(ii) *In aqueous solution*, whereby the cutting is stood in a dilute solution for periods of about 24 hr. This method is the commonest, and hitherto regarded as the most reliable.

(iii) *In a dust* (*e.g. talc*), an easy instantaneous method which requires relatively large amounts of growth substance, but which is not always reliable, one responsible factor being the variable size of the inert dust particles.

(iv) *In a concentrated alcoholic dip*, which is a more recent method and one gaining in favour. It involves instantaneous dip in a relatively concentrated solution of the growth substance in 50% alcohol, and is quite reliable. After treatment, the base of the cutting dries off, leaving a fine deposit of the substance. Another virtue of the dip technique is that it avoids what many regard as undesirable, the standing of the cutting in water for an extended time, and incidentally is quite independent of uptake conditions; furthermore, the effective dip concentrations appear to be much lower than at first appeared necessary (Hatcher & Garner, 1948).

The concentration of the growth substance to apply is determined by
(*a*) the plant to be propagated;
(*b*) the growth substance used;
(*c*) the method of application; and
(*d*) the time of year when the cutting is planted.

To this may be added another probable factor, the portion of stem used as the cutting. Here emerges a further advantage of the dip method, for the concentration giving optimal stimulation appears to have a lesser likelihood of being deleterious than with the older solution method.

Factors influencing rooting response

The factors controlling the behaviour of the cutting may be considered in the following categories:
(*a*) Species and variety.
(*b*) Culture relations of parent plant.
(*c*) Portion of shoot used and its size.
(*d*) Occasion of planting, and planting environment.
(*e*) Growth substance effect.

(*a*) The inherent regenerative capacity of the particular species is a primary factor, and while all plants will probably propagate if establishment is effected before separation, this is not so in the more severe process of cutting propagation, there being every degree of behaviour from the most difficult species to those which 'root like weeds'.

(*b*) Within the species or variety there is also considerable variability in rooting capacity. No better example can be found than that of fruit-tree rootstocks, those selected clonal races which form the basis of fruit-tree raising; for the demands of a highly specialized industry have occasioned intensive study of propagative behaviour, which is all the more interesting in that the fruit tree typifies the most difficult woody plants to propagate. To sum up many years of research it may be said that the performance of the cutting reflects the cultural conditions of the parent plant with reference to such factors as age of parent, closeness of plant, the general nutritional status of the parent, the method of culture, and the seasonal environment.

(*c*) Linked with differences of culture of the parent are the more localized effects of position in the shoot from which the cutting is taken, and also to some extent its size.

(*d*) Occasion of planting and planting environment form another complex of factors, in turn fundamentally influenced by geographical site. The tropical plant is quite a different proposition from the temperate one, the former experiencing no marked seasonal change, the latter manifesting pronounced phases of growth with deciduous habit. In the temperate climate cuttings are taken at two distinct times, (i) a leafy softwood cutting during the growing season itself, (ii) a leafless hardwood cutting during the dormant period. The softwood cutting is perhaps more easily propagated, but demands a heated frame; the hardwood cutting is normally planted in the open ground without expensive apparatus, this being the practical ideal, though not necessarily the optimal environment of the cutting. Within these two broad divisions there are further behaviour ranges, so that the potential planting season for hardwood extends from October to March. In this factor group are included the effects of the planting medium.

(*e*) Upon this interacting factor system was imposed the root-promoting action of growth substances. Far from providing a universal panacea for the propagator, their introduction has really added one further variant to complicate the already involved position. Instead of a straightforward investigation of growth-substance action, the wider problem has to be approached in a comprehensive manner, taking into account the main groups of operating factors.

East Malling propagation studies

Such a comprehensive approach is being made at East Malling, and the principles outlined are illustrated by the experimental results already obtained with the rootstock varieties, Crab C apple, a very difficult rooter even by the layering method, and Myrobalan B plum, a moderate rooter raised commercially from hardwood cuttings. These researches on rootstock propagation have passed into a new phase, the earlier ones being the investigation of the effects of external factors on cutting behaviour with some attention to internal factors local to the cutting, but not general to the parent plant as a whole; and growth-substance studies to investigate root-promoting power. The present phase shifts emphasis to factors operating through the parent plant, for whatever treatment or environment is given the cutting after 'separation', source factors have already operated to 'stamp' the cutting with a permanent impress of its past. The effect of growth-substance treatment on this depends upon such factors as nutritional status, anatomical features, and various metabolic circumstances, but within the framework set by the source environment of the cutting, however, there is ample evidence of the stimulating action of the synthetic growth substance.

Action of the growth substance on the cutting

Various workers, after a first growth-substance treatment, have removed the treated base and applied a second treatment, to determine if the effect of the latter is in any way modified by the former. If the effect of the growth substance is purely local on the tissues then no such modification would be expected; if, however, the second dose has a much smaller effect it would seem legitimate to argue that the initial dose causes the movement of internal substances into the base, which, being removed with it, are not available to the influence of a subsequent application of growth substance.

Results have been variable. Cooper (1936), using hardwood lemon cuttings, found a second treatment to have no effect, but Pearse (1938), using dormant willow cuttings, found the second treatment quite as effective as the first, as did Dorfmüller (1938), Hitchcock & Zimmerman (1938) and Verleyen (1948) with other species. In a later paper Cooper (1938) gives some results of auxin assays showing that the distribution of applied indole-3-acetic acid was similar in both apple and lemon cuttings, though only the lemon cuttings rooted, this supporting the idea of essential internal substances lacking in the apple, presumably not of the auxin type. Furthermore, Bouillenne & Bouillenne (1938), in experiments with hypocotyls of *Impatiens*, found that the stimulative effect of indole-3-acetic acid could be simulated by treatment with sugar solutions containing glycine, alanine and vitamin B_1, and concluded that the applied auxin speeded up root formation by mobilizing other internal substances. Nevertheless, Dittweiler (1942), in experiments with stem internodes of *Coleus*, showed that treatment with indole-3-acetic acid increased the amounts of natural auxin in the tissues, a process demonstrated to be independent of carbon assimilation, or even the presence of leaves and buds. But whatever their precise nature, it would seem on balance that such internal substances are affected by external supply of auxin, and in those cases where a second growth treatment following removal of the basal region receiving a first treatment is just as effective, it may not be justifiable to conclude the effect to be completely local. Thus Pearse's results with willow may have been due simply to slow reaction in the dormant tissues, and though Verleyen (1948) obtained a similar result with leafy cherry laurel cuttings, even here the first treatment may not have produced its effect before the treated basal region was removed. Clearly, the time factor must be taken into full account when comparing successive treatments of the same cutting, and it is interesting to note that the instantaneous treatment of the concentrated dip must necessarily depend for its action on an after effect.

Auxin analysis of the shoot and cutting

In conclusion reference should be made to the desirability of studying the natural auxin relations both of the shoots used as a source of cutting and of the cuttings themselves. Such an investigation is in progress at East Malling with the rootstocks already mentioned. During growth, both Crab C apple and Myrobalan B plum shoots release large amounts of free auxin from isolated stem sections, but as autumn approaches this disappears (Hatcher, 1948). It is still possible, however, to go on extracting auxin with ether as solvent. In the plum, where good rooting results from cuttings throughout autumn and winter, and from a considerable length of stem (Hatcher & Garner, 1949), it has been possible to extract auxin consistently from all parts of the shoot. In the apple, with its much lower rooting capacity, a shorter effective cutting season, and great propagative superiority of the base of the shoot (Garner & Hatcher, 1948), auxin could be extracted only up to the end of December, and then mostly from the basal region. As for the cuttings after planting, up to early March no auxin was extractable from Crab C, while auxin has always been extractable from Myrobalan B. This parallelism between regenerative behaviour and natural auxin is receiving further attention.

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FRUIT DROP IN THE APPLE IN RELATION TO SEED DEVELOPMENT

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In the apple, as in many other fruits, seed development and fruit development are closely correlated, a fact which the older pomologists explained in a general way by saying that seeds attracted nutrients to the fruit. In the light of modern physiological knowledge it seemed probable that a hormone mechanism was involved, and that the most likely source of this hormone was the developing seed. It has, in fact, proved possible to prepare from apple seeds at certain stages of development extracts which show hormonal activity, i.e. will induce parthenocarpy in the tomato and delay the abscission of delaminated petioles of *Coleus*.

Method of preparing extracts

The seeds, immediately on extraction from the fruit, are dried at 120° C. and ground to a fine powder. A weight of powder corresponding to 500 seeds is boiled in water for 15 min. and the water extracts shaken with four changes of peroxide-free ether. The ether washings are taken to dryness and the residue dissolved in 1 ml. distilled water. From this solution a series of dilutions are prepared for testing.

Test method

The test method used depends on the ability of the active substance to stimulate parthenocarpy in the tomato. It consists in adding a known quantity of the extract to a series of unpollinated tomato ovaries and measuring the resulting growth after a period of 6 days. By comparing the growth with that induced by known quantities of a synthetic growth substance (2-naphthoxy-acetic acid) it is possible to express the hormone content of the seed as microgram equivalents of 2-NOA.

Results with the variety Beauty of Bath

The hormone content of the seed, the size of the seed and the rate of fruit drop were measured at approximately weekly intervals from petal-fall to harvest. Two main peaks in hormone production were observed at approximately 30 and 75 days after petal-fall respectively. Hormone was first detected in the seed on the 23rd day, its appearance coinciding with the cessation of the first wave of fruitlet dropping. The 'June' drop was not observed in this variety, but there was a heavy pre-harvest drop which coincided with the final disappearance of hormone from the maturing seed. In two other varieties which showed a typical 'June' drop (Miller's Seedling and Cox's Orange Pippin) it was shown that the hormone content per seed was lower in those fruitlets which abscissed than in those which remained attached to the tree. The number of seeds per fruit was also lower in the apples which dropped.

A study of the internal development of the seed showed that the fluctuations in the hormone content throughout the season were correlated with the development of the endosperm. The first peak of hormone production occurred at the time when the endosperm passed from the free nuclear to the cellular condition. Subsequent fall in hormone production corresponded to the period of rapid embryo growth, when the endosperm was being digested faster than it was regenerated by meristematic activity. The second peak on the hormone curve occurred at the time the embryo attained its maximum growth. At this time, both the amount of endosperm and the amount of hormone in the seed were at a maximum and there was no fruitlet shedding.

The separation and individual extraction of the different tissues of the seed confirmed that the bulk of the hormone was present in the endosperm tissue, though significant amounts were also present in the embryo.

Results with the variety Lane's Prince Albert

Preliminary investigations with this variety have confirmed and extended the results obtained with Beauty of Bath. The first wave of fruitlet abscission again occurred during the period when the endosperm was free-nuclear and was terminated as soon as hormone appeared in the seed. This first peak of hormone production was not nearly as marked as in Beauty of Bath, and its effect on fruit drop was of short duration, the first drop being followed closely by the 'June' drop. The termination of the June drop coincided with a period of very active hormone production which occurred between 8 and 10 weeks after petal-fall. This major peak on the hormone curve appeared, in this variety, to be associated with embryo abortion, a phenomenon which was observed in approximately 80% of the seeds. Abortion of the embryo at an early stage of development resulted in seeds containing an exceptionally large proportion of endosperm tissue. Later, the endosperm tissue itself collapsed and hormone production ceased.

Conclusions

The tentative conclusion reached from these experiments is that fruit drop in the apple is under the control of a hormone produced in the endosperm of the seed, and that the successive waves of fruit drop are correlated with temporary deficiencies in hormone production resulting from developmental changes in the endosperm. In particular, the first drop seems to correspond with the period before the endosperm has become organized as a cellular tissue, and the 'June' drop, when it occurs, with the period of rapid embryo growth when the endosperm is being absorbed more rapidly than it can regenerate by meristematic activity. The cause of the pre-harvest drop is not yet clear. It may be correlated with a degeneration of the endosperm in the maturing seed but other factors too are probably operative.

Regarding the part this hormone plays in fruit growth, as opposed to fruit drop, little can be said at the moment. It appears to play no part in the initiation of fruit development, but there is some evidence that it may affect the later stages of growth.

REVIEWS

Conditioned Reflexes and Neuron Organization. By J. KONORSKI. Cambridge University Press. 18*s*.

The present century has seen the fruition of the work of two outstanding investigators of the mode of action of the central nervous system, namely Sherrington and Pavlov. Sherrington put forward a general conception of the working of the central nervous system which has been highly stimulating and fruitful, and which primarily consists in considering its action as a function of the organization of its neurones. Konorski points out that the problems raised by Sherrington's great book have been studied mainly with reference to the reflex activity of the spinal cord and subcortical ganglia, with the result that immense advances have been made in knowledge of minute structure as the basis of the activity of the nervous system. But the problems immediately outstanding in this field are now becoming exhausted and interest and emphasis is thus shifting to the higher centres of the cerebral cortex. In this field there has been comparatively little advance since the time of Sherrington's monograph. Pavlov initiated and contributed very extensively to an investigation of the activity of the cerebral cortex by means of the conditioned reflex method. As a result he has developed a theory of nervous processes entirely different from Sherrington's, and based exclusively on the results of the conditioned reflex technique. Pavlov's theory has nothing to say about the micro-structure of the nervous system and involves an entirely different set of concepts such as 'radiation', 'concentration', 'inhibition' and 'induction'. The present work is dedicated to Pavlov and to Sherrington, and its object is to bridge the gulf between their respective achievements. The author is uniquely fitted for his task. He worked for many years with Pavlov in Leningrad and regards Pavlov as his teacher. After leaving Pavlov's laboratory he continued his investigations in his own laboratory, the Nencki Institute of Experimental Biology in Poland. Although at first greatly influenced by Pavlov's genius and by his methods and scientific conceptions, he came more and more to see how fundamentally inadequate was Pavlov's theory, and how increasingly impossible it becomes to reconcile it with the evidence from the general physiology of the central nervous system which has been built up all over the world as a result of Sherrington's lead. He feels that the time is ripe to submit Pavlov's theories to intensive criticism and to reinterpret the vast store of experimental results accumulated by his school in the light of the work of Sherrington and his collaborators in the hope of producing a theory which would be more compatible with the general principles of neuro-physiology.

The work opens with an excellent summary of Pavlov's work, followed by an original and penetrating criticism of his theories. This section is sufficient by itself to make the book of value. According to Konorski the 'original sin' of Pavlov's theory is its assumption that fundamental plastic processes take place at the very beginning of the cortical part of the reflex arcs. This is why in Pavlov's writings the reflex arc as a whole disappears and we are left with the unspecified states of excitation, inhibition, etc., referred to above. Konorski's view is that it is the excitation of the unconditioned, not the conditioned centre, which is the chief feature. This brings back the whole subject into the terms of reflex arc and synapse, thus reverting once more to a neuron theory, whereas Pavlov's view is essentially anti-neuron. Konorski also points out how Pavlov turned his back on all attempts at psychological understanding and deliberately rejected introspection, although, in fact, it must remain one of the most valuable of tools.

A detailed criticism of the work would be out of place in this *Journal*. We can, however, say that on the whole the author has been remarkably successful in demonstrating the inadequacies of Pavlov's theories and what is more important in reinterpreting Pavlov's data, so

that it can be assimilated to present-day theories of neurone organization. This is not to imply that there are not certain conclusions and suppositions of Konorsky which are not open to criticism. It is, for instance, doubtful whether Konorski's morphological concept of plasticity in the nervous system can be harmonized with that of Lashley, J. Z. Young and other recent workers. This theory also leads to a number of discrepancies in conditioned reflex phenomena, particularly with relation to the basic properties of inhibition. Thus on pages 174–5 we are led to assume that since we cannot accept the concept of inhibition of inhibitory synapses, inhibition of the inhibitory reflex must take place in one of its intermediary excitatory relays. Konorsky is also open to criticism over his terminology and what he calls conditioned reflexes of the second type. He himself stresses the fundamental difference between the classical conditioned reflex and this second type, and even goes so far as to say that they must be regarded as a 'separate kind of plasticity'. He gives a brilliant account of the distinction between these two types of behaviour, but does not seem fully to realize that his conditioned reflex of the second type is nothing more nor less than 'trial-and-error learning' in its purest form. Having thus made so abundantly clear the difference between the two, it is surely merely adding to confusion to continue to use the term 'conditioned reflex' for both. As long as this term is confined to the classical conditioned reflex, it has a definite and valuable meaning, but when it is used as widely as in this work, it becomes almost meaningless and this is particularly unfortunate when there is the already perfectly good and well-defined term 'trial and error' available. Similar objections might also be raised against his use of the fantastic term 'dynamic stereotype'. Although the author seems to regard this (page 204) as a useful explanatory hypothesis it seems very doubtful if it can be the explanation of anything in the sense of which the word 'explanation' is used earlier in the book, for the term 'stereotype' does not connote any physiological idea or mechanical model. Naturalists and biologists working in the general field of animal behaviour will read with pleasure, tinged perhaps with amusement, the rather naïve statement that a classical conditioned reflex is not the only mechanism of plasticity and that 'the higher animals display other kinds of plastic changes, whose gradual recognition and investigation by objective methods is an important task of the physiologist of higher nervous activity'. If students of reflex conditioning had managed to keep in mind rather more than they have, the extraordinary variety and adaptiveness of behaviour as seen throughout the animal kingdom, we might have been saved much of the rather desiccated and academic discussion which has seen the light in the last thirty years. It is indeed welcome that Konorski has initiated a more biological trend, and it is greatly to be hoped that his work will be the means of bringing physiologists generally to take a broader view of the animals they study.

However, most of these criticisms are of a minor character and it remains to be said that this book is a great achievement and constitutes undoubtedly one of the most important recent works concerned with fundamental biological principles. As such, no physiologist concerned with nervous functioning and organization can afford to be without it. It is a profound and courageous work which marks a definite stage in the development of a theory of neuron activity.

W. H. THORPE

The Diseases of the Flax Plant (Linum usitatissimum *Linn.*) By ARTHUR E. MUSKETT and JOHN COLHOUN. Pp. 112. Belfast: W. and G. Baird, Ltd. 1947. 21*s.*

Flax Diseases. By R. MCKAY. Pp. viii + 55. Dublin: Flax Development Board, Ltd. 1947. 5*s.*

Until these two books were published, authoritative information on diseases of flax in the British Isles was to be found only by searching the various scientific journals for the original records of observation and research. Most of these records were largely accounts of work which had been stimulated by one or other of the two world wars.

Early in the first war, experience soon laid emphasis on the toll taken by disease, and investigations were encouraged by the Irish Department of Agriculture and Technical Instruction; interest had waned with peace-time conditions, to be revived with even more intensity in the second war, when the British Isles was once more dependent on its own production. Both books contain a distillate of the information assembled during the two periods.

The Diseases of the Flax Plant is not only a very readable survey of established facts but, in the words of the Minister of Agriculture for Northern Ireland, is a 'report of research work done by the Staff of the Ministry of Agriculture on behalf of the Flax Development Committee'. The first three-quarters of the book is devoted to accounts of diseases of cultivated flax known to occur in the British Isles. Each disease is considered separately, and special attention is paid to prevention and control. The authors are particularly qualified to write on the latter aspect as they have been primarily responsible for the outstanding success obtained in the prevention of disease by seed dressing. The last quarter of the text is made up of four appendices which are of particular interest to those concerned with the technique of seed treatment. In the first three of these is given a succinct account of the Ulster Method of seed examination for the detection of seed-borne parasites; of methods of disinfection and of the Northern Irish experience in the production, certification, storing and disinfection of seed in bulk.

Damage by two of the most serious diseases, Seedling Blight (*Colletotrichum linicola*) and Stem Break or Browning (*Polyspora lini*), was reduced to negligible proportions by the routine examination and treatment of all flax seed sown in the United Kingdom during the greater part of the second war. The most practicable method of treatment was by the application of a dust containing tetramethylthiuram disulphide. A third seed-borne disease, Foot Rot (*Phoma* sp.), did not become prominent until 1944 and this has not yielded to control by dry seed dressings. More success was obtained by the authors' adaptation of the Short Wet Method.

The fourth appendix, headed 'A Healthy Flax Crop', should help to maintain a balanced outlook and to put restraint on those enthusiasts who consider that the only way to control disease is by means of therapeutics. In this appendix, due emphasis is given to the effects of cultural methods on the incidence of disease. With regard to resistance, the authors remark that 'it is possible that the future prospects for flax breeding lie more in the direction of producing varieties resistant to disease than in attempting to increase the fibre yield'.

The format of the book deserves mention. After trying for some years to reconcile ourselves to books 'produced in complete conformity with the authorized economy standards', it is refreshing again to be able to turn over smooth glossy pages. The text is accompanied by abundant illustrations which portray symptoms of flax diseases, and many of the causative fungi are illustrated by micro-photographs. Also included is a useful series to show the character of growth of fungal colonies on agar as seen in the Ulster Method of seed testing. The illustrations much increase the value of the book, though the quality of reproduction is not uniformly good, especially where colour has been introduced. An index would have added to the usefulness of the book as a work of reference.

In his preface, the author of *Flax Diseases* states that 'the primary aim of this Bulletin is the recognition of flax diseases in the field and to bring some of the accumulated knowledge within reach of those interested in flax cultivation'. This aim is achieved by the presentation of the theme in an easy style supported by fifty-two half-tone photographs and a section on insect pests for good measure. For the agricultural officer and for the fieldman the book should provide a ready help to the identification of disease and also give him an appreciation of the way in which the parasitic organisms cause loss.

In considering methods of control, it is clear that the writer has not observed the same successes from the application of seed dressings under conditions in Eire as were experienced in Ulster and in Great Britain. Thus, although he found that seed dressings reduced the ravages of Seedling Blight, he points out that the disease was not at all unusual in Eire in 1945

when all seed had been dressed with a substance containing tetramethylthiuram disulphide, and he has had little or no success to record in the control of Stem Break and Browning (*Polyspora lini*). For control of this disease he recommends the hot-water treatment of seeds. His analysis of the failure to control by external dressings is given in the final section of the book headed 'General Observations'. He remarks that 'out of the twelve fungi mentioned as causing more or less disease on the crop, six have been demonstrated as being carried internally in the seed...'.

The book, which has a stiff paper cover, is of a size which allows it to be carried in the pocket of the average overcoat and its remarkably low price places it within the reach of the most junior fieldman. H. H. GLASSCOCK

Scientific Horticulture. The Journal of the Horticultural Education Association. Norwich: Jarrold and Sons Ltd. Vol. IX, pp. 1–178. 1949. 10*s*.

The first volume of *Scientific Horticulture*, then called the *Horticultural Education Association Year Book*, was published in 1932. Thereafter it appeared annually until 1939, the title being changed to *Scientific Horticulture* in 1935. The 1939 number was Vol. VII; it is intended that Vol. VIII shall consist of the five 'Occasional Publications' which appeared during the years 1939–47, thus linking the pre-war issues with the present volume. The decision to bind the issue in a cloth cover and offer it for sale to the public will be welcomed, especially by non-H.E.A. members.

The volume consists largely of a series of papers by specialists in the various branches of horticulture, its main theme being the first post-war refresher course in horticulture held in September 1946; these are supplemented by articles written in the next two years covering recent progress in some aspects of the subject. The volume should be particularly useful to those unable to take the refresher course and will serve as a very useful reference and handbook on the many and increasing facets of horticulture which now have to be studied by grower and adviser alike.

Any attempt to review all the aspects of the subject dealt with in this volume would necessitate the help of many workers and the reviewer must therefore be content to mention the contributors and the titles of the articles only. These are as follows: H. V. Taylor, Horticulture in the N.A.A.S.; A. Muir, Soil Surveys; C. Bould, Soil Organic Matter and Composts; W. J. C. Lawrence, Recent Work with Seed and Potting Composts; C. E. Elms, Recent Developments in Horticultural Machinery; O. Owen, Tomato Nutrition; D. J. D. Nicholas, Rapid Tissue Tests for Mineral Nutrients in Plants; W. A. Roach, Plant-Injection Methods; W. G. Templeman, Soil-less Culture; G. Fox Wilson, The Specific Uses of DDT and Gammexane in Horticulture; H. Martin, Recent Developments in Insecticides and Fungicides—Part I; W. C. Moore, The Incidence of Plant Diseases in England and Wales; M. B. Crane, Genetics applied to Horticulture; M. A. H. Tincker, The Hormone Concept in Relation to Horticulture; G. E. Blackman, Selective Weed Control in Horticulture; F. Glover, Certification Schemes for Growing Plants; H. G. H. Kearns & O. G. Dorey, Some Reflexions on Machinery for Fruit Production; P. H. Brown, Some Reflexions on Machinery for Vegetable Production; J. Rhodes & E. E. Skillman, Irrigation of Horticultural Crops; H. Martin, Recent Developments in Insecticides and Fungicides—Part II; R. B. Dawson, Selective Weedkillers for the Control of Weeds in Turf; M. L. Yeo, Fuel Utilization in Horticulture.

The articles are illustrated by twelve plates with twenty-nine excellent photographs and three text-figures. The volume richly deserves a very wide circulation, and it is unlikely that future numbers will need to carry advertisements which appear to be out of place in a publication of this nature. I. THOMAS

Chemistry of Insecticides, Fungicides and Herbicides. By DONALD E. H. FREAR. Pp. x+417. New York: D. van Nostrand Company, Inc.; London: Macmillan and Co. 2nd ed. 1948. 33*s*.

The second edition of this text-book will be welcomed by all students and teachers of agricultural chemistry for, in the six years since the first edition, progress in the discovery and utilization of chemicals effective for pest control has raced ahead with ever-increasing speed.

Dr Frear's book is a 'graduate course dealing with the chemistry' of these compounds, and is written not only for the chemist but for the biologist whose 'attempts to control insects and plant diseases would be facilitated by a better understanding of the chemistry' of the products used. In the first nineteen chapters of the book he describes those chemical and physical properties of the inorganic and synthetic organic insecticides, the natural organic insecticides, fungicides and herbicides, which determine the biological properties and manner of use of these materials. He has written these chapters with a skill and certainty that prompt the wish that the subject-matter had not been confined to graduate level. But a discussion of the still largely hypothetical views of the mode of action of these compounds would be out of place in a book intended primarily for students.

By the same token, however, the final two chapters dealing with analytical methods seem inadequate. These methods are reprinted from the 'Official and Tentative Methods of Analysis' of the Association of Official Agricultural Chemists and from an article published fourteen years ago in these *Annals*. They were written for the qualified analyst and incorporate the detail and refinements required of standard methods. Given without an explanation of the analytical reactions involved or of the many steps taken to avoid interference by impurities, this section of the book is far above the heads of students who surely should never be asked to work blindly to the printed word but should be told the reason for each step of the analytical procedure. A point of less moment is that the methods have not been brought up-to-date; for examples, the factor for Pyrethrum I by the mercury reduction method is now 0·0057 and not 0·0044; the original methods proposed for the analysis of tar-oil preparations have been improved in Bulletin 122 of the Ministry of Agriculture.

H. MARTIN